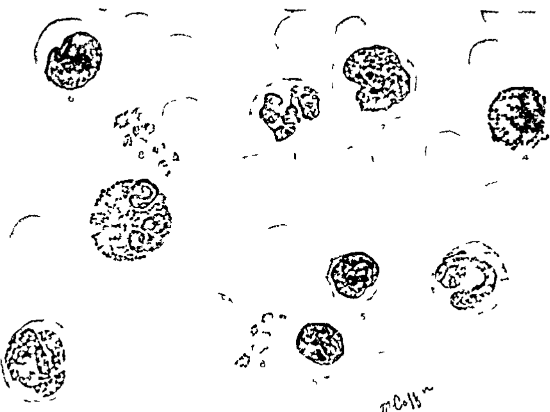


NORMAL CELLULAR CONSTITUENTS OF ADULT HUMAN BLOOD

1 Segmented (polymorphonuclear) neutrophil 2 Band (stab) neutrophil 3 Segmented eosinophil 4 Basophil 5 Small lymphocytes 6 Large lymphocyte 7 Monocytes 8 Thrombocytes and 9 Erythrocytes (From Custer An Atlas of the Blood and Bone Marrow)

Fundamentals of

CLINICAL HEMATOLOGY

By

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PREFACE

KEEPING ABREAST of the literature devoted to hematology is a formidable task although monographs textbooks special journals devoted to original contributions review articles and abstracts are available to those who are interested However the pleasant task of teaching medical students over a period of years has led us to conclude that there is a need for a new textbook that is designed for the student who is being introduced to hematology for the first time while he is in the midst of a crowded curriculum Physicians in active practice similarly pressed for time have expressed a desire for a similar book one that is comprehensive but not encyclopedic that could serve for a review and reorientation in the field

This book has been written in an effort to fulfill these needs Emphasis is placed on a consideration of the abnormal mechanisms that are responsible for the manifestations of the different diseases A unifying approach of this type can provide a foundation for further development by those who are interested in the subject It can also provide a sound basis for the management of hematologic problems that are seen in clinical practice

A presentation of the morphologic and functional characteristics of the various cell lines is followed by discussions of the normal physiology of erythropoiesis leukopoiesis and hemostasis In each instance the current theoretical considerations are discussed with particular reference to their

application in diagnosis and treatment The discussions of most of the clinical syndromes are accompanied by short accounts of actual cases which illustrate appropriate points Special consideration has been given to the diagnosis of patients who present the problems of anemia hemorrhagic diathesis or lymphadenopathy A separate chapter is devoted to hematologic techniques

The authors express their appreciation to the following members of the Faculty of the School of Medicine of the University of Virginia who generously helped us in many ways particularly by their suggestions and criticisms Drs William Parson Thomas Hunter Charles Crockett Donald Shotton J T Carpenter and William Herring of the Department of Internal Medicine Drs O B Bobbitt and Cecil Hougie of the Department of Clinical Pathology Drs Morris Lambdin and Audrey Brown of the Department of Pediatrics Dr George Cooper Jr of the Department of Radiology Dr David Smith of the Department of Pathology Dr D R H Gourley of the Department of Pharmacology Dr R W McGilvery of the Department of Biochemistry Dr Edward Cawley of the Department of Dermatology also to Dr Allan Sharp of the Radcliffe Infirmary United Oxford Hospitals Our thanks also go to Miss Barbara Marshall Miss Peggy Parker Miss Annie Lipscomb Mrs Ralph Fitzwater Mrs Charles Mahon and Mrs Anna Dabney for their help in preparing the material and to Miss Anne Russell and Mrs Hope Ansbacher of the Department of Medical Illustration who prepared many of the illustrations for us The authors are also grateful for the help and encouragement they have received from the editorial staff of the W B Saunders Company

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CONTENTS

The Morphology, Origin, Chemistry and Function of Blood Cells

THE IMPORTANT contributions made by Ehrlich can be considered the beginning of modern hematology[43] The staining methods which he introduced became the cornerstone of morphologic hematology and cytochemistry With the use of the new technique Ehrlich described many cellular details and developed a classification of the blood cells He recognized that the granulocytic series of cells arose from a non granular precursor in the bone marrow and thought that lymphocytes arose from the lymph "glands" This distinction formed the basis of his theory of the dual origin of the white cells

Although Ehrlich considered this dualistic doctrine to be one of the most important results of his long continued studies in hematology[43] this theory was not accepted by many hematologists and numerous controversies followed The dualistic theory was supported by Banti Turk Naegeli and others[43, 9] and was opposed by the monophyleticists Maximow Jordan Bloom and others who believed that myeloid tissue could arise postem

lus has been correlated with active cellular proliferation[67, 153] According to Thorell cellular proliferation ceases when the nucleolus disappears and is replaced by the more deeply basophilic heterochromatin[131]

Myeloblasts vary in size and other details The nucleus is sometimes slightly indented and in acute leukemia it is occasionally deeply indented or lobulated The cytoplasm of myeloblasts and monoblasts sometimes contains Auer bodies These structures which appear red with Wright s stain and resemble a stained tubercle bacillus give positive peroxidase and oxidase reactions, Histochemical study has revealed that these rods contain ribonucleic acid and a lipoid material possibly a mucopolysaccharide[1] When myeloblasts are stained supravitally with neutral red and Janus green numerous small mitochondria can be seen in the cytoplasm Recent studies have shown that the mitochondria which may contain a complete Krebs cycle are a source of succinoxidase and cytochrome oxidase activity[6, 33]

2 *Progranulocyte (Promyelocyte)*

The progranulocyte is the next stage in development after the myeloblast and some may resemble myeloblasts with the addition of azurophilic cytoplasmic granules Other cells at this stage have a coarser nuclear structure than the myeloblast are quite large and contain numerous azurophilic granules in the cytoplasm and in the region overlying the nucleus Nucleoli may or

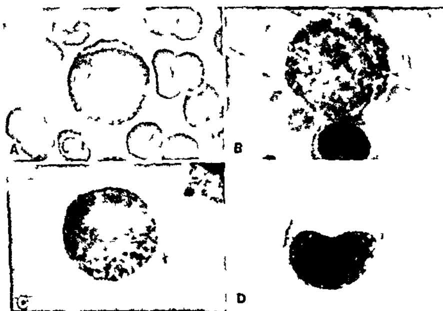


Figure 1 Early cells of the granulocytic series A Myeloblast B Promyelocyte C Myelocyte (early) D Metamyelocyte

bryonically from lymphocytes.[16, 9] Still another theory, the polyphyletic, has been advanced by Cunningham, Sabin, and Doan, who concluded that each type of adult cell has its own particular blast cell precursor.[79] Detailed discussions of the different theories of the origin of the various cells are presented in Downey's Handbook of Hematology[41] and in review articles.[1, 9, 91, 116]

Despite an enormous amount of study the question of the origin and interrelationship of the cells remains unsettled after more than half a century. Much of the confusion regarding the identity of cells has been caused by the lack of a uniform terminology. Different techniques and different types of material have been used by investigators who have given different names to the same cells and have employed the same term for different cells. Fortunately, unanimity of hematologic opinion as to the origin and relationship of the cells is not essential for the successful management of most clinical hematologic problems. There are enough points of agreement to allow the development of the workable concept of cellular relationships that is needed for an organized approach to clinical problems. A knowledge of morphology is necessary for the diagnosis of many hematologic diseases and a terminology is essential for communication.

In selecting the terms to be used in this text the authors have chosen those that are used most commonly in the classification of cells; the terms recommended by the Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood Forming Organs have been included in every instance.[3]

I. THE GRANULOCYTIC SERIES

MORPHOLOGY

1. *Myeloblast*

The myeloblast occurs in normal bone marrow but is not found in the circulating blood except in certain disease states. In fixed smears the diameter of myeloblasts ranges from 11 to 18 microns. With Wright's stain the nuclear material assumes a deep purplish blue color; the chromatin is finely divided, punctate or strandlike, and appears condensed only around the rim of the nucleoli. Several light blue staining nucleoli are usually visible. The non-granular cytoplasm, which stains the same light blue color as the nucleolus, is variable in amount and often forms little more than a rim about the nucleus. Myeloblasts generally give a negative reaction when a peroxidase stain is used, but some cells that are classed as myeloblasts with Wright's stain give a positive reaction with peroxidase stain. The staining properties of the cells are related to the nucleoproteins.[1,3] Ribonucleic acid (RNA) occurs both in the nucleoli and in the cytoplasm, while deoxyribonucleic acid (DNA) is found only in the nucleus. Protein synthesis in the cell is thought to be under the control of the nucleoproteins and the presence of the nucleo-

lus has been correlated with active cellular proliferation [80, 1, 3] According to Thorell cellular proliferation ceases when the nucleolus disappears and is replaced by the more deeply basophilic heterochromatin [131]

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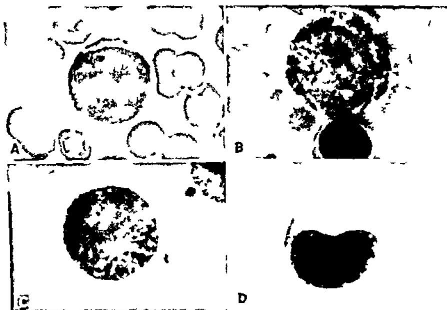


Figure 1 Early cells of the granulocytic series *A* Myeloblast *B* Promyelocyte *C* Myelocyte (early) *D* Metamyelocyte

may not be visible at this stage The appearance and development of the granules appear to be closely related to the differentiation of the cytoplasmic proteins Thorell has shown that the myeloblast has a high concentration of cytoplasmic polynucleotides and a prominent nucleolus[131] When the cell matures to the progranulocytic stage the cytoplasmic polynucleotides decrease as the granules appear Further development of the granules is associated with continued decrease in the polynucleotide content and none is demonstrable by the time the myelocyte stage is reached The nucleolus which has been thought to have considerable influence over the differentiation of the cytoplasmic proteins contains ribonucleic acid this material decreases gradually and is no longer detectable in the myelocytic stage When the nucleolus disappears further differentiation of the cytoplasmic protein cannot take place[80]

3 *Myelocyte*

Myelocytes are the first cells of the granulocytic series that contain specific granules which are either neutrophilic, eosinophilic or basophilic Both neutrophilic and eosinophilic granules give a positive peroxidase reaction As the myelocyte develops from the progranulocyte it becomes smaller and the nuclear chromatin becomes coarser When supravital stains are used numerous small mitochondria and a variable number of neutral red bodies can be demonstrated in the cytoplasm The number location and arrangement of the neutral red bodies have been employed as a basis for classifying myelocytes according to different stages of development as myelocytes A B and C [9]

4 *Metamyelocyte (Juvenile)*

As the myelocyte matures the chromatin structure of the nucleus becomes coarser specific granulation persists and the nucleus becomes indented At this stage the cell is termed a metamyelocyte

5 *Band Cell (Stab Cell)*

According to the Committee on Nomenclature of Blood Cells any cell of the granulocytic series is classed as a band cell which has a nucleus which could be described as a curved or coiled band, no matter how marked the indentation if it does not completely segment the nucleus into lobes connected by a filament"[3]

6 *Segmented Cell (Polymorphonuclear)*

These are the fully developed cells of the granulocytic series whose nuclei contain two or more lobes that are joined by filamentous connections The nuclear chromatin is coarse and has the appearance of blocks or knots

connected by strands The cytoplasm contains numerous granules that stain specifically with Wright's stain

The neutrophil segmented cell (neutrophil) gives a positive peroxidase reaction In supravital preparations numerous small refractile bodies fill the cytoplasm and active ameboid movement is prominent At times neutrophils are unusually large and have nuclei consisting of five to ten lobes Such cells termed macropolycytes or segmented cells pernicious anemia type" occur most often as a result of a deficiency of vitamin B_{12} or folic acid

The eosinophil segmented cell (eosinophil) is a fully developed cell The nucleus does not stain as heavily as that of the neutrophil The eosinophil is characterized by the presence of large granules that fill the cytoplasm and stain a bright yellowish red color with Wright's stain. When stained supravitally with neutral red and Janus green the granules appear large very refractile and yellow These cells give a positive peroxidase reaction Histochemical studies indicate that the eosinophil granules are composed of protein and surrounded by phospholipid [14] Verdoperoxidase is also present [3] Eosinophils contain about one-third of the histamine found in normal blood [58]

The basophil segmented cell (basophil) is somewhat smaller than the neutrophil and eosinophil The characteristic feature of this cell is the presence of large purplish black granules that appear to almost completely fill the cell The granules appear red when stained supravitally with neutral red The cells are peroxidase negative Basophils contain relatively large

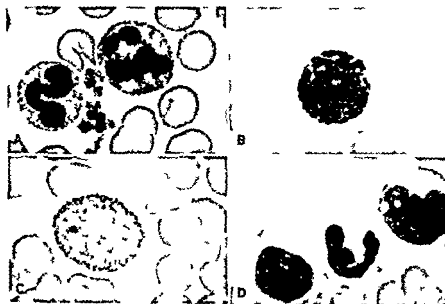


Figure 2 Later cells of the granulocytic series A Neutrophils B Basophil C Eosinophil D Comparison of lymphocyte neutrophil monocyte

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enzyme in the granulocytes has been found to be increased in infection and in polycythemia and decreased in myelocytic leukemia It has been reported that alkaline phosphatase activity parallels glycogen concentration[14] Neither the function of this cellular enzyme nor the mechanism underlying the variations that have been found in various disease states is known[8, 137]

4 *Acid phosphatase* activity has also been demonstrated in the leukocytes Unlike alkaline phosphatase which is confined mainly to the granulocytes[137] acid phosphatase occurs in all types of leukocytes The acid phosphatase activity of the leukocytes is high in chronic myelogenous leukemia and normal in the leukocytosis of infection

Among the other substances that have been identified in leukocytes are histamine[1] which is confined largely to the granulocytes[1] (predominantly the basophils[138]) dipeptidases tripeptidases[51] sulfhydryl groups[9, 63] lipase and maltase[93] beta glucuronidase[3] peroxidase[9] and glucuronic acid[5] Phospholipids are present in the granules in granulocytes[14]

FUNCTION OF THE GRANULOCYTES

*Although the recognition of a large number of enzymes and other chemi*cal substances in granulocytes suggests that these cells have multiple functions at the present time the primary function of the leukocyte is considered to be concerned with the defense of the body against various noxious agents The importance of phagocytosis as one of the main defense mechanisms of the body was suggested by Metchnikoff in 1882 after he had observed the accumulation of mobile cells about a thorn placed under the skin of the star fish larva[100] The stimulus responsible for the movement of the leukocyte into the proximity of the offending particle is believed to be chemical in nature and is termed chemotaxis while the actual engulfment of the particle by the leukocyte is termed phagocytosis

Chemotaxis is said to be positive if the leukocyte moves toward the particle negative if repelled and indifferent if it is unaffected by the presence of the particle The term is applied only to the direction of movement and not to the speed of movement[81] Numerous investigators have tried to identify the specific substance or substances responsible for chemotaxis It has been demonstrated that crystalline nitrogenous material from inflammatory exudates attracts leukocytes in large numbers when placed in the cutaneous tissue of an animal[87, 84, 6, 8] This substance which has been termed leukotaxine appears to be a polypeptide[6] It has also been suggested that the attracting substance is glycogen[73]

After the granulocyte has been attracted to the site phagocytosis occurs The neutrophil is more adept than the eosinophil or basophil at this operation The younger granulocytes bands and metamyelocytes are more avid than the segmented cells in attacking bacteria in the presence of opsonins[12]

Various factors influence phagocytosis It appears to be enhanced in some types of anemia[17] possibly as a result of pteroylglutamic acid deficiency[10] and by an increase in temperature from 0° C to 35 C[40] It is reduced by

amounts of histamine [0 1 138] about one half of the histamine content of normal blood [90] The presence of heparin has been suspected but not proved

ORIGIN OF THE GRANULOCYTES

Under normal conditions the older granulocytic cells the bands and segmented cells develop from the myelocytes in the bone marrow Various cells have been considered to be the non granular ancestors of the granular myelocytes [9 63 9 9] Although these theories differ in details all agree that in the adult under normal circumstances the older cells of the granulocytic series arise in the bone marrow and are derived from the myelocytes and their precursors which are either myeloblasts or cells that have an identical appearance in fixed smears

CHEMISTRY OF THE GRANULOCYTES

Advances in biochemical and histochemical methods have revealed interesting information about the chemistry and function of the granulocytes Although the functional significance of many of the enzymes and chemical substances that have been identified in the granulocytes is as yet unknown a brief discussion is presented in order to indicate the extent and complexity of this rapidly expanding field Many of the studies have been concerned with various aspects of carbohydrate metabolism Some have been undertaken in an effort to determine if metabolic pathways in normal granulocytes differ from those found in certain diseases particularly leukemia [7]

1 *Glucose*[1 5] and other hexoses[71] are metabolized by the granulocytes to lactic acid Immature forms have a predominantly oxidative metabolism and do not form lactic acid under aerobic conditions while mature forms have high lactic acid production uninfluenced by the presence of oxygen [0] The presence of the intact cellular membrane is believed important to the metabolic activity of white blood cells[8] because in broken cells absolute respiration drops to one half and the glycolytic rate to but one fifth that of intact cells Several enzymes and a number of phosphorylated intermediates[14 146] important in glucose metabolism[146] have been identified Recent work suggests that glycolysis may follow more than one pathway in the mature leukocyte [7 78]

2 *Glycogen* has been demonstrated in the cytoplasm of granular leukocytes and its concentration is thought to increase as the phagocytic and ameboid activities of the cell increase [77 143] The glycogen content of the leukocytes from children with glycogen storage disease has been reported to be increased [144] The possibility has been suggested that the leukocytes act as a transport system for delivery of glycogen to areas where it is needed [143]

3 The determination of *alkaline phosphatase* activity which has been demonstrated in both normal and abnormal cells of human blood and bone marrow may prove useful in clinical hematology [140] The activity of this

and the identity of a blast cell usually is assumed from the recognition of the older cells that accompany it The nucleus of the lymphoblast is round and has a fine chromatin structure in which several nucleoli are often visible The cytoplasm stains pale blue and contains no granules

2 *Prolymphocyte*

Lymphocytic cells that are intermediate between lymphoblasts and lymphocytes are termed prolymphocytes Although the nuclear chromatin appears coarser than that of the blast cells it is finer than that of the lymphocytes At times nucleoli or nucleolar remnants are visible Usually the diameter of these cells is greater than 15 microns

3 *Lymphocyte*

In dry smears the diameter of small lymphocytes varies from 7 to 10 microns but the less common large lymphocytes may have a diameter of 20 microns When Wright's stain is used the nucleus of the small lymphocytes which is usually round but sometimes indented appears almost to fill the entire cell The nucleus has a prominent membrane stains deeply and contains large masses of chromatin No nucleoli can be identified In the large lymphocytes the nuclear chromatin stains less deeply and appears somewhat less compact The cytoplasm which is much more abundant in the larger lymphocytes stains pale blue A small number of reddish violet granules are scattered through the cytoplasm of many of the cells of this series The lymphocytes do not give a positive reaction with the peroxidase stain When stained supravitally with Janus green and neutral red the lymphocytes are seen to contain a variable number of short rodlike mitochondria that usually appear clumped in one part of the cytoplasm often opposite the nuclear indentation A few small neutral red vacuoles are also seen

Lymphocytes at times show definite staining abnormalities in disease states particularly in virus infections The cytoplasm of the larger cells is



Figure 3 Lymphocytes *A* small *B* large

high concentration of glucose,[73] and by malnutrition.[8] The application of agents which alter the surface charge of the cells or the particles alters the phagocytic activity.[1] These results are in agreement with other observations[95,30] that relate phagocytosis to surface charge. However, other results seem to indicate a chemical relationship between the particle and the cell.[94,97,98]

The important role that the properties of the particles to be engulfed play in the process of phagocytosis is demonstrated by studies that utilized inert solid particles[48] and bacteria.[163] Many bacteria produce antiphagocytic substances which are toxic for the phagocytic cell.[163] Among these are the somatic "O" antigen on the surface of many gram negative species, exotoxins and leukocidins. The growing type III pneumococcus resists phagocytosis in a different manner. It produces a polysaccharide "slime" layer composed of long chained carbohydrate polymers of glucose and glucuronic acid which protects the organism from phagocytosis and contributes to the virulence of this organism.[1,7]

The enhanced phagocytosis of organisms which occurs in the presence of immune sera and complement is known as the opsonic function of the antibody.[163] The manner by which opsonin renders microorganisms more susceptible to destruction is incompletely understood. In the absence of opsonin phagocytes are unable to engulf bacteria floating free in a liquid medium. In such circumstances cells can phagocytize organisms after they have succeeded in trapping them against a convenient surface, such as the wall of an alveolus or another leukocyte. This process has been termed surface phagocytosis.[1,6,18]

Studies in patients with cyclic neutropenia and in patients with persistent agranulocytosis associated with asplastic anemia indicate that the granulocytes play a minor role at most in defense mechanisms other than phagocytosis.[101] Such studies indicate no influence on antibody production, the febrile response to endotoxin administered intravenously, or the local reactions to endotoxin or bacterial allergy. The responses to appropriate stimuli in the form of increased production of C reactive protein and mucoprotein appear to be unimpaired in the absence of granulocytes. Perhaps the most significant observation in such patients concerns the deficiency not only of granulocytes but also of lymphocytes in cutaneous inflammation, which suggests that the neutrophilic granulocytes (or a product of their reaction with the tissue) afford the stimulus for the migration of lymphocytes to the area of inflammation and their subsequent transformation into macrophages.

II THE LYMPHOCYTIC SERIES

MORPHOLOGY

1 *Lymphoblast*

The lymphoblast occurs in the peripheral blood rarely if at all except in cases of leukemia. It is distinguished from the myeloblast with difficulty

and the identity of a blast cell usually is assumed from the recognition of the older cells that accompany it The nucleus of the lymphoblast is round and has a fine chromatin structure in which several nucleoli are often visible The cytoplasm stains pale blue and contains no granules.

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Figure 3 Lymphocytes *A* small *B* large

sometimes deeply basophilic and opaque and at other times it is almost colorless Nuclear fenestrations and cytoplasmic vacuoles are often present

ORIGIN OF THE LYMPHOCYTES

Lymphocytes are produced in lymph nodes in the periarterial pulp of the spleen, in nodules of the tonsils in the mucous membranes of the gastro intestinal, genitourinary and respiratory tracts and possibly in the lymph nodules in the bone marrow [41, 6, 9, 116] Various cellular origins of the lymphocytes have been suggested Among these are preexisting lympho cytes [16, 6] reticulum cells [16, 39, 116] hemocytoblasts [16, 6] and "primitive white cells intermediate between reticulum cells and lymphoblasts [9] Much evi dence suggests that the lymphocyte is not an irreversible end cell" such as the granulocyte but instead is a cell that retains the potential for further differentiation at least under special circumstances The lymphocyte has been considered to be the source of plasma cells[79, 1, 9] and also of wandering tissue phagocytes [9, 101] The conclusion has also been reached by some in vestigators that lymphocytes can produce myeloid cells[39, 6, 66] and erythro- cyte precursors[63, 66, 9] under the specific stimulus of special environmental factors

CHEMISTRY OF THE LYMPHOCYTES

Most of the studies on the quantitative and qualitative chemistry of the leukocytes have been performed on mixtures of granulocytes and lympho cytes What is known of the chemistry of the lymphocyte has been deter mined largely by the application of histochemical methods By such methods a number of enzymes and other chemical substances of uncertain function have been identified in the lymphocytes Acid phosphatase[137] and glycogen[110] have been identified in the lymphocytes in the circulating blood These cells have been found to contain more dipeptidase and less tripeptidase than the granulocytes [1] When the lymphocyte migrates to a site of inflammation its morphological change into a macrophage is accompanied by changes in its cytochemistry [110] These are the acquisition of oxidase and peroxidase activ ity the appearance of alkaline phosphatase and an increase in sudanophilic cytoplasmic constituents These changes occur when the lymphocyte is un dergoing hypertrophy at which time there is both a relative and an absolute increase in mitochondria

FUNCTION OF THE LYMPHOCYTES

The function of the lymphocyte in the circulation and in other tissues throughout the body continues to be the subject of considerable contro versy It has been considered by many to be an adult cell perhaps more vestigial than functional because it was thought to possess neither nucleoli nor powers of movement Others have always considered it to be a young

cell capable of forming other cells normally or in the times of stress The observation that enormous numbers of lymphocytes enter the circulation each day via the thoracic duct the visualization of nucleoli by means of supravital stains and the newer types of microscopy, the recognition of a characteristic type of motility and the demonstration that these cells contain a large amount of deoxyribonucleic acid have all combined to produce a reawakening of interest in lymphocytes [167] At the present time most of the interest and controversy related to the lymphocyte is concerned with its possible function as a hematopoietic cell or as a vital part of the defense mechanism of the body

The ability of even small lymphocytes to form other lymphocytes homoplastically is considered established by some because of the demonstration of mitoses in material from lymph nodes bone marrow and spleen [110] However *mitoses are seen much more often in large and medium sized lymphocytes than in small lymphocytes in the germinal centers of lymph nodes* The discrepancy between the large number of lymphocytes present in the body and the infrequent occurrence of mitosis in lymphoid tissue may be explained by a heteroplastic origin from reticulum cells and by a long life span of at least twenty one days as measured by studies with DNA labeled with radioactive phosphorus [110] Yoffey has calculated that for every lymphocyte normally present in the circulation three lymphocytes enter the circulation each day from the thoracic duct and 23 are present in the bone marrow [162] The origin of the marrow lymphocytes is thought to be hematogenous rather than myelogenous The function of the large number of lymphocytes contained in the marrow but not organized into lymphoid tissue remains an unsettled problem According to one concept these are resting primitive cells able to be transformed into blast cells of various types including erythrocyte precursors and thus the main function of the small lymphocyte is cellular production [6, 167] It has also been suggested that the large number of lymphocytes in the tissues and their reaction to adrenal cortical steroids indicate that these cells have a role in protein metabolism [1] (2)

The role of the lymphocyte in defense depends in large measure on its ability to be transformed into other cells such as macrophages and plasma cells The lymphocytes possess limited but definite power of phagocytosis. However transformation of lymphocytes into macrophages at a site of tissue inflammation is accompanied by a marked enhancement in phagocytic ability [110] Macrophages, which arise also from monocytes and histiocytes of the connective tissue are probably the most powerful phagocytes in the body It has been suggested that the small lymphocyte is simply a small edition of the macrophage and that those in the circulating blood constitute a reserve of potential macrophages [145]

The lymphocyte is also concerned with antibody formation Results obtained with the fluorescent antibody technique are consistent with the hypothesis that a small amount of antibody is formed in lymphocytes [5] However these studies show that the major portion of the antibody formation takes place in plasma cells and it is possible that the most important

function of the lymphocyte in this connection is in the production of plasma cells Lymphocytes may be transformed into plasma cells directly or indirectly by becoming histiocytes which in turn develop into plasma cells[116] The lymphocyte is assigned to still another role in antibody production in the reutilization hypothesis [6] Antibodies are not self replicating and it has been suggested that the transfer of immune reactions by the lymphoid tissue occurs because the nucleic acids of the lymphocytes which provide templates for the proteins concerned in antibody synthesis act as mediators when large fragments of the nucleic acids of small lymphocytes are phagocytized by reticulum cells which later become transformed into new lymphocytes or plasma cells [6] It has also been postulated that the initial step in antibody formation is the phagocytosis of the antigen by macrophages which become transformed into plasma cells and basophilic staining lymphocytes both of which possess the capacity for antibody synthesis The heteroplastic type of antibody formation which includes the concept of reutilization of antibody containing cells or components of these cells apparently predominates in active immunization A homoplastic type of antibody production the passing on to daughter cells of increments of antibody by proliferation of antibody containing lymphocytes (which may also involve a reutilization process) may be important in certain circumstances such as the proliferation of lymphoid tissue and sensitization by the transfer of antibody containing cells [1]

Views of the lymphocyte that are contrary to those presented above have been expressed by Gowans Gowans states that the small lymphocyte has long life span perhaps measured in months and its transformation into another cell type either in the bone marrow or in areas of inflammation has not been demonstrated unequivocally From his own studies he concludes that the large number of small lymphocytes that enter the venous system from the thoracic duct each day are cells that have recirculated from the blood Failure of others to find any significant DNA production in small lymphocytes in radioautographic studies that utilized tritiated thymidine which is incorporated solely into DNA supports the concept that the small lymphocyte does not possess significant proliferative activity [1, 6]

At the present time it is apparent that many important details of the lymphocyte particularly the small lymphocyte remain uncertain and controversial

III THE MONOCYTIC SERIES

MORPHOLOGY

1 *Monoblast*

According to the Committee on the Nomenclature of Blood Cells (1949) the monoblast is any cell of the monocytic series that has a fine chromatin structure [3] Nucleoli are usually visible, and such cells found in association with monocytes should be tentatively classed as monoblasts These cells do not occur in the peripheral blood or bone marrow under normal conditions

but may be seen in patients with monocytic leukemia In such circumstances many of the blast cells appear identical with the myeloblast

2 *Promonocyte*

Promonocytes are cells that have an indented nucleus and appear younger than the *monocytes* in that the nuclear chromatin appears *more* finely divided and contains nucleoli or nucleolar remnants These cells are thought to be intermediate between the blast cells and adult monocytes They are not seen in the peripheral blood normally but at times do occur in disseminated tuberculosis and other diseases involving the reticuloendothelial system as well as in monocytic leukemia

3 *Monocyte*

The monocyte is usually larger than the other cells of the peripheral blood In dried smears the diameter measures from 16 to 22 microns When stained with Wright s preparation the monocyte possesses an abundant pale bluish gray cytoplasm which appears somewhat more opaque than the cytoplasm of the lymphocytes The cytoplasm contains numerous fine reddish blue granules and vacuoles are often present The nucleus which is often but not invariably indented has a stringy chromatin structure that appears more condensed where the strands of chromatin meet When these cells are stained supravitally with neutral red and Janus green, large numbers of short mitochondria can be seen in the cytoplasm *Neutral red regularly stains a group of cytoplasmic vacuoles near the indentation of the nucleus This* rosette of red vacuoles has been considered by some authors to be a characteristic peculiar to monocytes [118] but others do not share this opinion [61] Although monocytes are usually easily distinguished from lymphocytes and myelocytes by their size and the appearance of the nucleus and cytoplasm at times atypical cells are seen that cannot be identified with confidence by any of the staining methods

ORIGIN OF THE MONOCYTES

In 1924 Maximow stated that of all the cells in the circulating blood the monocytes offer the greatest difficulty to elucidation of their relationship and origin [9] Three decades later both their origin and their relationship to the histiocytes (macrophages) remain uncertain At various times *monocytes* have been considered to arise from lymphocytes [16, 63] endothelial cells [76] histiocytes [9] myeloblasts [9] and monoblasts [118]

A relationship between monocytes histiocytes and lymphocytes *is shown* by the study of the reaction in inflammation Maximow [9] *considered it proved* that the tissue macrophages (polyblasts mononuclear phagocytic elements) arise partly through direct transformation of the free histiocytes of the tissue by mobilization and also through changes that occur in monocytes and lymphocytes that migrate from blood vessels into the field of inflammation

buck and Crowley who recently published an extensive review of the rk on this subject studied the cellular reactions occurring in sterile in mmation of the human skin and concluded that the main source of acrophages in the inflammation is the emigrated lymphocytes while the onocytes and tissues cells play a subsidiary role because of their limited mbers[109]

CHEMISTRY OF THE MONOCYTES

Sufficient numbers of monocytes have not been obtained for any quantitative chemical determinations However histochemical methods have revealed the presence of nucleic acids glycogen[14] a dehydrogenase lipi and phospholipids[14] The monocytes do not contain alkaline phosphatase b do contain acid phosphatase, oxidases and peroxidases[14] Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) are also present[18 111]

FUNCTION OF THE MONOCYTES AND MACROPHAGES

The monocyte is considered to be a young cell that is capable of many biologic activities[133] Normally it develops into a macrophage an occurrence which indicates that the monocyte has great potentialities for growth development of enzyme systems, phagocytosis and digestion Recent studies that utilized tritium labeled thymidine indicate that monocytes in the circulation are capable of synthesizing deoxyribonucleic acid an observation that suggests that the cell is destined to divide[17] It is not known whether this activity in a circulating cell indicates merely a capacity to propagate itself or the wider capacity possessed by a totipotential mesenchymal cell of fixed tissue The monocyte has the ability to synthesize protein and it has been suggested that these cells produce antibodies and other substances that are released by cytoplasmic shedding and cellular disruption[33 113] Others who believe that antibody production occurs almost entirely in the plasma cells and to a small extent in lymphocytes consider that the evidence fo antibody production by monocytes is indirect or negative The monocyte and macrophages possess great capacity for phagocytosis and in certai disease states they respond to specific stimuli by the formation of epithelioi cells and multinucleated giant cells[1 3] The monocyte responds to the presence of the tubercle in a characteristic fashion Multinucleated cells a formed and are believed to be the epithelioid cells of tuberculosis[117]

IV THE THROMBOCYTIC SERIES

MORPHOLOGY

1 *Megakaryoblast*

Megakaryoblasts which are found only in the bone marrow in special circumstances are larger than the other blast cells The cytoplasm is pale

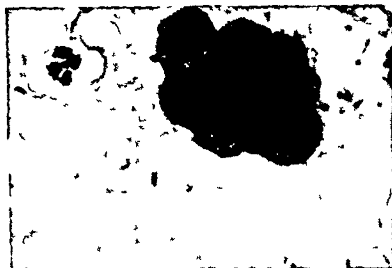


Figure 4 Megakaryocyte

blue The nucleus which is round or oval has a finely divided chromatin structure and a variable number of small nucleoli may be apparent

2 *Promegakaryocyte*

Promegakaryocytes are considered to be intermediate between megakaryoblasts and megakaryocytes The nucleus which has a shape similar to that of the megakaryocyte contains nucleoli surrounded by a rather coarse chromatin structure The cytoplasm contains a number of fine purplish red granules

3 *Megakaryocyte*

The megakaryocytes do not occur in the peripheral blood normally but are found in the bone marrow They are very large cells that often have a diameter that varies from 50 to 100 microns The nucleus which is usually multilobed stains dark blue with Wright's stain and contains no visible nucleoli The abundant cytoplasm stains light blue and contains numerous purplish red granules These granules often appear in aggregates

4 *Thrombocyte (Platelet)*

Thrombocytes or blood platelets are the smallest stained elements in the peripheral blood Ordinarily they have a diameter of from 2 to 4 microns but in some disease states and on smears made from blood collected with

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IV THE THROMBOCYTIC SERIES

MORPHOLOGY

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(ethanolamine phosphatide) that is essential in the first phase of blood coagulation

V THE PLASMACYTIC SERIES

MORPHOLOGY

1 *Plasmablast (Myeloma Cell)*

Plasmablasts do not occur normally in the peripheral blood or in the bone marrow and are seen only in plasmacytic leukemia or multiple myeloma The nucleus which is usually eccentric, has a fine chromatin structure and contains one or more large nucleoli The cytoplasm stains blue and is more opaque than that of the myeloblast or lymphoblast

2 *Proplasmacyte*

These cells are intermediate between the plasmablasts and the plasmacytes The chromatin of the nucleus appears more coarse than that of the plasmablast but is definitely more immature than that of the plasmacyte Usually a single nucleolus is present but at times more than one are seen

3 *Plasmacyte (Plasma Cell, Marschalko s Plasma Cell)*

In fixed smears the plasma cells vary considerably in size and diameters range from 10 to 20 microns The characteristic feature of the cell is the eccentric location of the round or oval nucleus which has extremely coarse chromatin The chromatin consists of blocks which stain deeply The arrangement of the chromatin in the nucleus has been compared in appearance to the spokes of a wheel The cytoplasm which often has an irregular border is abundant stains dark blue and is characterized by a perinuclear area of lighter staining The cytoplasm is non homogeneous and small vacuoles are often present When supravital stains are used numerous small mitochondria can be seen in the cytoplasm of the cell

Plasmacytes are not usually seen in the peripheral blood but may appear in serum sickness rubella, infectious mononucleosis, infectious hepatitis multiple myeloma plasma cell leukemia, and other conditions Normally plasmacytes are present in the lymph nodes, the connective tissue and the bone marrow

ORIGIN OF THE PLASMACYTES

Michels reviewed the rather extensive literature on the plasma cell that appeared prior to 1931 and noted that at least four different cellular origins had been suggested[89] These were (1) histogenous origin from connective tissue cells including tissue lymphocytes fibroblasts resting wandering cells (2) origin from emigrated lymphocytes (3) origin from emigrated lympho

an anticoagulant the platelets appear much larger In such circumstances they may approach the small lymphocyte in size or may form a strand that stretches across an entire oil immersion field These elements contain no nuclei and when stained with Wright's stain appear to consist of a pale cytoplasm that contains numerous reddish purple granules

ORIGIN OF THE THROMBOCYTES

Megakaryocytes are thought to arise from primitive mesenchymal cells by way of the hemocytoblast that is the precursor of the megakaryoblast[13]

Wright's hypothesis of the origin of the blood platelets as fragmented portions of the cytoplasm of the megakaryocytes of the bone marrow is generally accepted[101] More recent studies that utilized antisera[9] and the electron microscope[4, 64] support the same conclusion

CHEMISTRY OF THE THROMBOCYTES

The chemistry of the megakaryocytes and the platelets has been investigated extensively The cells are metabolically active and have a considerable turnover of phosphorus[1, 1] Peptidases nucleotidase acid phosphatase and alkaline phosphatase occur in substantial amounts The granulations of both thrombocytes and megakaryocytes are polysaccharides Ribonucleic acid (RNA) is present only in the megakaryocytic nucleus[111, 1, 8] Investigation of the lipid content of platelets has revealed that the largest percentage of the total lipid fraction consists of phospholipid 68 per cent of which is cephalin[45] It is interesting that the platelets contain the same amount of lipid by weight as the leukocytes Dehydrogenase activity which ordinarily disappears with the destruction of the integrity of the cell structure is present in the platelet[7] Platelets contain pyrophosphatase which is liberated before evidence of platelet damage becomes apparent[31] Cytoplasmic nucleotides are present in the megakaryocytes and are broken down as the cell grows older The finding of their degradation products in the platelets is interpreted as evidence that the older cells are responsible for the formation of platelets[39]

FUNCTION OF THE THROMBOCYTES

The chief function of the platelet is in hemostasis However there is increasing evidence that the platelet like other formed elements in the blood may serve as part of a transport system that carries enzymes or other chemicals from one part of the body to another

In hemostasis the platelet serves in both a mechanical and a chemical role It acts mechanically to seal defects in the vascular endothelium and also to promote clot retraction by its influence on the strands of fibrin Platelets contain serotonin (5 hydroxytryptamine) and may act chemically to promote vasoconstriction of the small vessels They exert a more important influence on blood coagulation by releasing a substance (probably

cytes or preexisting tissue lymphocytes (4) origin from immature blood cells such as myeloblasts or erythroblasts, through aberration Sundberg has recently reviewed the subject of the origin and function of the plasma cells[1 9] and pointed out that while studies on the production of antibodies emphasize a close relationship between the plasma cells and the lymphocytes the concept of multiple origins of plasma cells from lymphocytes, perivascular cells mesothelial cells macrophages and fibroblasts, which was proposed by Downey in 1911 cannot be excluded with certainty [4]

CHEMISTRY OF THE PLASMACYTES

Although both ribonucleic acid and deoxyribonucleic acid have been identified in plasma cells the small number of these cells that are available has limited the study of their chemical composition

FUNCTION OF THE PLASMACYTES

Since the discovery of antibodies by von Behring in 1890 a great body of data has been accumulated as the result of attempts to find the site of their production The lively discussions which have resulted[17] may be due in part to the confusion arising from the morphologic similarities of the young lymphocyte and plasma cell and to the variations in experimental methods used by various investigators Recently the evidence that the plasma cell is the major site of antibody production has become almost indisputable [4 5 70] It has been demonstrated that a marked increase in plasma cells occurs in the spleens of animals receiving repeated injections of antigen tissue explants from the spleens of these animals continue to produce antibody in vitro These tissue explants contain plasma cells By means of the fluorescein labeled antibody technique it has been shown that the antibody develops in response to the second injection of antigen The production of antibody in the plasma cells is associated with a marked increase in the number of plasma cells

The plasma cell is only rarely phagocytic [19] and the various inclusion bodies that sometimes occur in the cytoplasm are probably formed intra cellularly

VI THE ERYTHROCYTIC SERIES

MORPHOLOGY

The terminology that has been used in discussions of the nucleated cells of the erythrocytic series has been the source of considerable confusion Because different definitions have been given to the same term by different authors the Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood Forming Organs has recommended an entirely new terminology for the nucleated cells of the erythrocytic series [23] The terms recommended—rubriblast prorubricyte rubricyte and metarubricyte—have not been used widely The Committee felt that the recommended

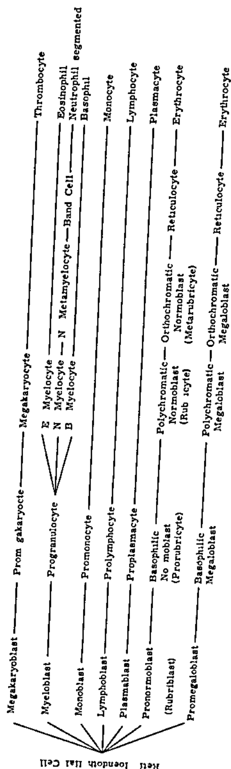


Table 1 Origin and Relationship of Blood Cells

— Mc

Mc

M

names indicated stages of differentiation in development of the adult erythrocyte. They recommended that the qualifying phrase "pernicious anemia type" be used to indicate the cells that showed the morphological characteristics seen in patients with untreated pernicious anemia.

Usually the type of erythropoiesis is described as being either "normoblastic" or "megaloblastic." The term "megaloblast" has been used with different meanings in the literature. Ehrlich introduced the term to indicate the large nucleated precursor of the erythrocytes that is characterized by its much weaker affinity for nuclear stains.[43] He thought that the presence of this cell indicated an abnormal type of erythropoiesis that was seen in adults almost exclusively in pernicious anemia, and that it represented an embryonic type of cell. He considered it a line of cell maturation different from the "normoblastic" type which was seen in nearly all other types of anemia. The normoblastic type of maturation was characterized by erythrocyte precursors that contained a nucleus that gave an intense color with nuclear stains. Doan, Cunningham, and Sabin used the term "megaloblast" to indicate the first generation of cells in the red blood cell series that could be distinguished from endothelial cells in either the embryonic or adult types of erythropoiesis.[34] Thus, according to one usage of the term, "megaloblast" indicates an abnormal type of erythropoiesis that occurs only in special disease states when seen in postnatal human beings; according to the other usage the term indicates a very young erythrocyte precursor that may occur whenever erythropoiesis is markedly increased but otherwise normal. The term "erythroblast" has been used by some authors to refer to all forms of nucleated red corpuscles, but Doan, Cunningham, and Sabin have used this term to indicate an intermediate stage in the development of the erythrocyte.[34] The term "megaloblast" is used in this text as it has been used by Ehrlich, Downey, Jones, and Wintrobe.[41, 43, 1]

Erythropoiesis is considered as being normal in type when the normoblastic series is present and abnormal when the megaloblastic series is present. Similar stages in the development of the nucleated red cells can be recognized in both series. In normoblastic maturation the cells of the different stages are termed pronormoblast, basophilic normoblast, polychromatic normoblast, and orthochromatic normoblast. In the megaloblastic type of maturation the corresponding cells are termed promegaloblast, basophilic megaloblast, polychromatic megaloblast, and orthochromatic megaloblast.

1. *Pronormoblast (Rubriblast)*

The pronormoblast is the youngest recognizable precursor of the erythrocyte. Its diameter varies from 12 to 19 microns. The nucleus, which is round,

Figure 5 (opposite page — upper). Normoblastic hyperplasia in hemolytic anemia.

Figure 6 (opposite page — lower). Megaloblastic hyperplasia in pernicious anemia. (Both figures from Heilmeyer, L., and Begemann, H.: Atlas der klinischen Hämatologie und Cytologie. Springer, 1955.)

present Both of these staining reactions are signs of immaturity and reflec the persistence of ribose nucleoprotein in the cells Punctate basophilia (stippling, is increased after exposure to lead and other metals

In disease states the erythrocytes are often abnormal The cells may sho

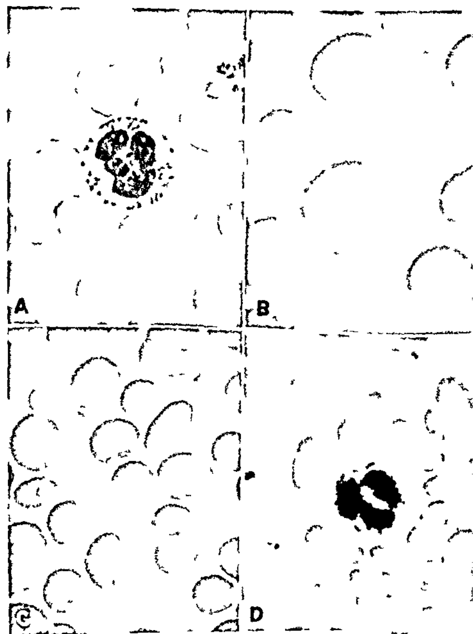


Figure 7 Erythrocytes in peripheral blood Wright's stain A Normal erythrocytes B Macrocytes C Hypochromic microcytes D Ovalocytes

certain conditions catalase functions as a peroxidase and this may be the role it plays in the red blood cells [67]

Acetylcholinesterase has been the subject of widespread interest recently That which is present in the erythrocyte is firmly fixed to the stroma[10] and may influence the permeability of the cell to potassium and sodium ions [60] It may serve also in the protection of the body against excessive acetylcholine production The acetylcholinesterase content of reticulocytes and young red cells is increased and measurement of the enzyme in the erythrocytes can serve as an index of marrow activity [109, 118, 1-3] Markedly reduced activity of erythrocyte acetylcholinesterase has been demonstrated in patients with paroxysmal nocturnal hemoglobinuria

Carbonic anhydrase catalyzes the formation and dissociation of carbonic acid It makes possible the rapid removal of carbon dioxide from the tissues and its release from the lungs [115] The observation that this enzyme was partially inhibited by the sulfonamides led to the development of the more potent carbonic anhydrase inhibitors

A deficiency of red cell glutathione has been found in persons sensitive to primaquine [13] Administration of primaquine results in destruction of the older red blood cells perhaps as the result of oxidative damage to the hemoglobin or the stroma which is evidenced by the appearance of Heinz bodies The appearance of Heinz bodies will also result from the exposure of sensitive cells to acetylphenylhydrazine in vitro A deficiency of glucose 6 phosphate dehydrogenase has also been demonstrated in primaquine sensitive cells It has not been demonstrated that either deficiency is the primary defect in these cells but it has been postulated that glucose 6 phosphate dehydrogenase deficiency results in impaired triphosphopyridine nucleotide reduction which in turn results in a deficiency of reduced glutathione Patients sensitive to primaquine have been demonstrated to be sensitive to acetanilid furadantin sulfanilamide napthalene and other substances This defect is transmitted by a gene which is sex linked and has variable expressivity It occurs in approximately 10 per cent of Negroes and 1 per cent of Caucasians Fava bean sensitive subjects have also been demonstrated to have deficiencies of the same factors but other factors may be operative as well

Two enzymes have recently been isolated from mammalian erythrocytes which are believed to be concerned with maintenance of hemoglobin in a functional state Methemoglobin reductase[64] is a hemoprotein with a molecular weight of approximately 185 000 It has been postulated that together with reduced triphosphopyridine nucleotide and possibly reduced diphosphopyridine nucleotide methemoglobin reductase maintains the hemoglobin of the intact red blood cell in the reduced form Glutathione peroxidase[90] is a heat labile and non dialyzable enzyme isolated recently from mammalian erythrocyte hemolysates which catalyzes the oxidation of reduced glutathione by hydrogen peroxide It has been suggested that this enzyme functions with glutathione to protect hemoglobin from oxidative damage in the intact

The over-all aerobic reaction produces 38 moles of adenosine triphosphate and is nineteen times as efficient as the anaerobic pathway which results in but 2 moles of adenosine triphosphate

It has been shown that the erythrocytes consume minute quantities of oxygen If this represents true respiratory activity it could contribute to the energy production of the cell.[23, 127] This important point has not been settled but the aerobic mechanism appears unlikely because the mature red blood cell contains no mitochondria, which are thought to be essential to the aerobic pathway of glucose metabolism.[2, 127] The available evidence indicates that anaerobic glycolysis but not the tricarboxylic acid cycle is important for the metabolism of glucose by the erythrocyte The enzymes necessary for the operation of the phosphogluconate oxidative pathway are present in the red blood cell As a result of the operation of this shunt reduced triphosphopyridine nucleotide is made available in the red blood cell Ordinarily glucose is broken down through a series of reactions to lactic acid[6] The rate of glycolysis by the mature erythrocyte is from 0 3 to 0 4 mg glucose/cc of packed cells/hr The erythrocyte in contrast to other cells utilizing anaerobic glycolysis contains an unusually large amount of 2,3-diphosphoglycerate and no glycogen Some consider that 2 3-diphosphoglycerate serves as a carbohydrate storage compound in place of glycogen It is utilized when the supply of glucose to the cell is exhausted This substance is also an important catalyst in glycolysis[139]

During glycolysis for every mole of glucose degraded to lactic acid there is a net gain of two moles of ATP Hydrolysis of a mole of ATP to one mole of ADP and one mole of inorganic phosphate results in the release of the equivalent of 7000 calories The utilization of this energy in the red blood cell is poorly understood It may be concerned with the maintenance of the cellular membrane and with transport of materials across the membrane The mechanism by which ions and molecules enter and leave the erythrocyte has not been clearly defined in any instance but there is general agreement that an active process is involved[6] Insulin has not been demonstrated to play a role in the transport of glucose across the red blood cell membrane

With the exception of the enzymes that are involved in glycolysis catalase has perhaps been more extensively studied than the other erythrocyte enzymes It is similar to hemoglobin in its structure and is composed of a protein moiety and four iron containing heme groups All of the heme compounds—catalase hemoglobin myoglobin the cytochromes and peroxidase—have in common the porphyrin ring structure with complexed iron atoms but they contain different protein fractions All are involved in essential metabolic activities The synthesis of catalase remains at normal levels even when iron deficiency is severe enough to produce anemia The catalase content of the erythrocytes has not yet been shown to vary sufficiently in disease to be diagnostic of any particular disorder[11] Catalase is a potent catalyst for the decomposition of hydrogen peroxide The function usually ascribed to catalase is that of protecting hemoglobin against decomposition by hydrogen peroxide[67, 11] however it has been pointed out that under

certain conditions catalase functions as a peroxidase and this may be the role it plays in the red blood cells[57]

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erythrocyte Complete understanding of the role of these enzymes in erythrocyte metabolism must await further study

Ergothioneine[58a] and a number of enzymes including phosphatases[10 1 3] urease[1 8] peptidases[1] arginase[14] glyoxalase[43] lactic dehydrogenase[101a] and glutamic oxalacetic transaminase[1 8] have also been identified in the erythrocyte Whether they play a role in the metabolic life of the mature erythrocyte or are merely remnants of the metabolic functions of the normoblast is unknown It has also been suggested that the red blood cell may serve to transport these substances from one area of the body to another

The Stroma

The stroma is the insoluble portion of the erythrocyte which remains after the cell has been hemolyzed and washed free of hemoglobin The stroma is frequently referred to as a "ghost" It is composed of 40 to 60 per cent protein and 10 to 12 per cent lipids The lipid fraction consists of 60 per cent phospholipids 30 per cent free cholesterol and 10 per cent cholesterol esters and neutral fats[46] The free cholesterol is in dynamic equilibrium with the cholesterol of the plasma The blood group substances A and B and the Rh antigen are located in the stroma

FUNCTION OF THE ERYTHROCYTES

The function of the erythrocyte is to carry oxygen from the lungs to the cells of the body and to transport carbon dioxide to the lungs The hemoglobin molecule in the mature erythrocyte is in a stable state and there is no degradation or resynthesis during the life span of the cell However hemoglobin does change constantly from the reduced to the oxidized state When the iron is in the reduced form hemoglobin can bind and transport oxygen Ferric or oxidized hemoglobin which is known as methemoglobin is not able to function as part of an oxygen transport system As the reduced form is auto-oxidized to the ferric form special reactions are necessary to reduce methemoglobin as rapidly as it is formed These reactions function so efficiently that not more than 1 per cent of the hemoglobin in the erythrocyte is in the oxidized state It is probable that reduced triphosphopyridine nucleotide which arises from the phosphogluconate oxidative pathway and the enzyme methemoglobin reductase are mainly responsible for the reduction of methemoglobin[43 4 64 149]

Patients with congenital methemoglobinemia have a defect in the methemoglobin reductase mechanism leading to a concentration of methemoglobin much higher than normal and to secondary polycythemia[4] These patients have been successfully treated with methylene blue an auto-oxidizable dye which functions as an electron carrier This compound greatly accelerates glucose oxidation via the phosphogluconate oxidative pathway and the reduction of methemoglobin in the mature erythrocyte[64b]

An elevated level of methemoglobin is also found following the introduc

tion into the body of compounds which preferentially oxidize hemoglobin and exceed the capacity of the normal red blood cell reducing systems Among the most important offenders are nitrites, sulfonamides, and aniline derivatives[49] This secondary methemoglobinemia is usually mild but serious cell damage and hemolysis may occur Treatment with methylene blue is usually successful

Sulfhemoglobin is another compound which is not capable of carrying oxygen Its formation represents an irreversible change in hemoglobin and it persists until the destruction of the red blood cell It is unaffected by methylene blue or ascorbic acid Some source of sulfur such as a sulfur containing drug or chronic constipation plus the ingestion of a drug which will oxidize hemoglobin is thought necessary for its production The syndrome of enterogenous cyanosis is probably related to the formation of either methemoglobin or sulfhemoglobin secondary to disturbed bowel function[49]

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peripheral destruction At present it appears that tissue oxygen tension controls erythropoiesis this control being mediated by a humoral factor which has been named *erythropoietin* [11,31,74] Despite the efforts of numerous investigators the biochemical nature of this substance has not been characterized [8,3] Erythropoietin increases red blood cell production by increasing the rate at which cellular division proceeds in the marrow The rate at which the erythrocyte matures or is released into the circulating blood does not seem to be affected [3,67] If the stimulus is sufficiently intense the rate of erythrocyte production may be increased six to eight times normal [17]

Figures 8 to 11 indicate how a simple negative feedback system might operate to control erythropoiesis

The physiological control of erythropoiesis in the normal person assuming an adequate supply of necessary protein vitamins and minerals is depicted in Figure 8 The daily destruction of approximately 2×10^{11} erythrocytes must be compensated by the release of an equal number of erythrocytes from the marrow The location of the cells which produce erythropoietin in response to oxygen deficit is not yet known [7] though there is evidence which suggests that the kidneys are involved [49]

Hemorrhage or an increase in the rate of destruction of erythrocytes results in an anemia if uncompensated The total quantity of oxygen reaching the tissue cells is reduced because of the decreased number of cells An increase in erythropoietin activity of the plasma has been demonstrated under such conditions and is thought to be responsible for the increased erythropoiesis which compensates for the loss of red blood cells (Fig 9)

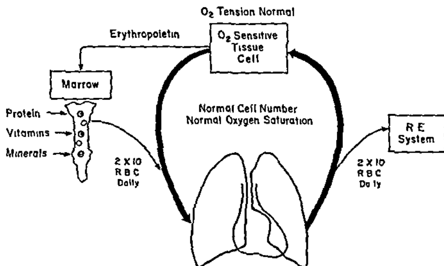


Figure 8 Schematic representation of the control of erythropoiesis under normal conditions

CHAPTER II

Erythropoiesis and the Metabolism of Hemoglobin and Iron

ERYTHROPOIESIS

THE PRIMARY FUNCTION of the red blood cell is the transport of respiratory gases In order to fulfill this function there must be sufficient circulating erythrocytes to meet the metabolic needs of the body There is a compensatory increase in the number of peripheral red blood cells when there is a decrease in the amount of oxygen reaching the tissue cells of the body If there is a surfeit of oxygen available there occurs a decrease in circulating erythrocytes

THE CONTROL OF ERYTHROPOIESIS

The physiological control of the number of circulating erythrocytes is maintained by regulation of erythropoiesis and not by regulation of their

peripheral destruction At present it appears that *tissue oxygen tension* controls erythropoiesis this control being mediated by a humoral factor which has been named *erythropoietin*[11,30,50,74] Despite the efforts of numerous investigators the biochemical nature of this substance has not been characterized[8,3] Erythropoietin increases red blood cell production by increasing the rate at which cellular division proceeds in the marrow The rate at which the erythrocyte matures or is released into the circulating blood does not seem to be affected[3,67] If the stimulus is sufficiently intense the rate of erythrocyte production may be increased six to eight times normal[2]

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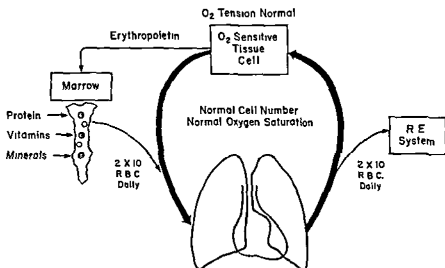


Figure 8 Schematic representation of the control of erythropoiesis under normal conditions

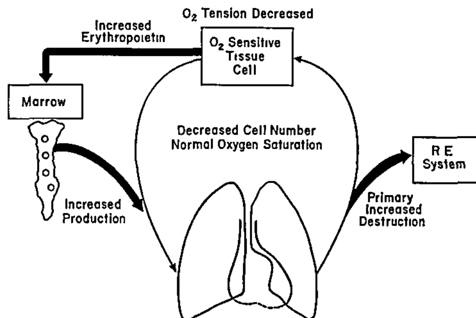


Figure 9 Schematic representation of the control of erythropoiesis with increased loss of red cells by bleeding or increased destruction

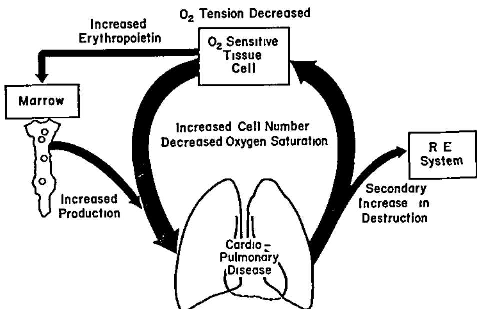


Figure 10 Schematic representation of the control of erythropoiesis in cardiopulmonary disease with decreased arterial oxygen saturation

Decreased arterial oxygen saturation resulting from cardiopulmonary conditions has been found to increase erythropoietin activity and to be associated with hyperactive erythropoiesis (Fig 10) A secondary polycythemia results from the sustained stimulus[1] A similar situation is thought to be obtained when cobaltous chloride is administered to a patient Increased levels of erythropoietin have been demonstrated following administration of cobalt[6] but the mechanism through which this effect is produced is unknown[4]

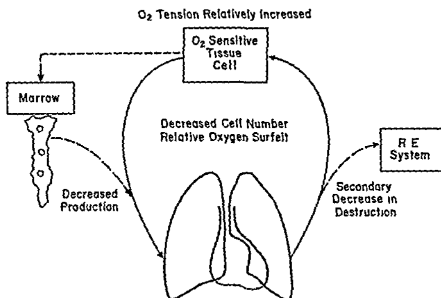


Figure 11 Schematic representation of the control of erythropoiesis under conditions of oxygen surfeit

Anemia is associated with hypothyroidism in a high percentage of cases Decreased oxygen demand by the tissue cells might be expected to be reflected by diminished erythropoietin production and anemia (Fig 11) The red blood cell count stabilizes at a level sufficient to meet the diminished metabolic demands of the body It has been shown however that further reduction of the circulating red blood cells by bleeding results in a prompt reticulocytosis with a return of the red blood cell count to the prebleeding level in a normal period of time These studies suggest that the anemia of myxedema occurs as the result of diminished metabolic demands rather than as an abnormality of the erythropoietic system

THE ROLE OF HEMOGLOBIN

Hemoglobin is formed by the developing erythrocyte in the bone marrow The most rapid hemoglobin formation is in the class III (polychromatophilic

normoblast) red cell when approximately 80 per cent of the hemoglobin carried by the mature cell is formed [100] Though hemoglobin may be formed in the reticulocyte and under exceptional circumstances the immature non reticulated erythrocyte its *formation under normal conditions is confined to* the developing normoblasts in the marrow [61, 10]

The level of hemoglobin in the blood represents the balance between production and destruction of the hemoglobin molecule In the normal person the hemoglobin level is constant as production and destruction are nicely balanced

The total quantity of hemoglobin in the blood may be determined by multiplying the concentration of hemoglobin by the blood volume since hemoglobin production and destruction are in a state of equilibrium If the concentration of hemoglobin is 15 gm per cent (mean male equals 16.3 gm per cent mean female 14.5 gm per cent) [1] and the total volume roughly 5000 ml the total quantity of hemoglobin is calculated as follows

$$\text{Total hemoglobin} = \text{concentration of hemoglobin (gm/ml)} \times \text{blood volume (ml)}$$
$$\text{Total hemoglobin} = 0.15 \times 5000 = 750 \text{ gm hemoglobin}$$

The life span of the hemoglobin molecule is approximately 120 days its destruction occurs coincident with that of the erythrocyte If the total amount *of hemoglobin in the body at any one time is 750 gm and the life span of* the red blood cell is 120 days the amount of hemoglobin formed each day can be calculated since 1/120 of the total amount must be formed daily

$$\frac{\text{Total hemoglobin}}{\text{life span in days}} = \text{rate of production/day}$$
$$\frac{750}{120} = 6.25 \text{ gm hemoglobin/day}$$

Under normal conditions the body produces approximately 6.25 gm of hemoglobin per day The maximum effort of the body as for example in the event of hemolytic disease has been calculated to be approximately 40 gm/day [16] This means that the survival time of the erythrocyte may be reduced to 18 or 19 days without the occurrence of anemia if the bone marrow functions at maximal capacity

$$\frac{\text{Total hemoglobin}}{\text{life span in days}} = \text{rate of production/day}$$
$$\frac{750 \text{ gm}}{X} = 40 \text{ gm/day} \qquad X = 18.8 \text{ days}$$

When hyperhemolysis is compensated excessive destruction of red blood cells exists without the development of anemia It is generally felt that the increase in the production of red blood cells and hemoglobin is brought about by increase in the mass of erythroid precursors rather than an increase in the rate of maturation in the individual red blood cell [19] though the latter has not been ruled out as a contributing factor

If the destruction of red blood cells exceeds the maximum effort of the bone marrow anemia develops The anemia becomes stabilized when eryth

rocyte production and erythrocyte destruction proceed at a constant rate. While the values found for hemoglobin and red blood cells are not normal they do not decrease progressively unless the life span of the cell is further shortened or unless there is interference with marrow activity. The laboratory findings of patients with sickle cell anemia illustrate these points. The life span of the erythrocyte is shorter than can be compensated for by maximal marrow activity and in usual circumstances the subnormal hemoglobin value stabilizes at a level that depends on the life span of the erythrocyte and the maximal marrow output. This anemia is made more severe by a sudden increase in hemolytic activity or by a decrease in erythrocyte production. Either mechanism results in a further reduction of circulating erythrocytes.

THE CHEMISTRY OF HEMOGLOBIN

Hemoglobin has a molecular weight of 66,700[1,4] and is thought to be ellipsoid in shape.[18,40] The results of x-ray crystallographic studies have indicated that the hemoglobin molecule is composed of two identical half molecules[70] and this has been confirmed by the analysis of tryptic hydrolysates.[41] Each half molecule has now been shown to contain two different peptide chains which have been designated α and β.[7] The organization of the approximately 300 amino acids which comprise the α and β peptide chains is under genetic control and their synthesis occurs early in the development of the normoblast.[2,3] The importance of the proper organization of the amino acids during the synthesis of these proteins has been demonstrated recently by comparing the molecular structure of the hemoglobin from normal persons with that obtained from patients with one of the hemoglobinopathies.[41]

Of the approximately thirty peptide spots obtained from heat- and trypsin-digested hemoglobin by electrophoresis and chromatography, only one peptide from sickle cell hemoglobin differed from normal.[41] Analysis of the abnormal peptide revealed that a glutamic acid residue had been replaced by valine.[4] A similar study of the C hemoglobin molecule indicated that the same peptide was again abnormal but in this instance the normal glutamic acid had been replaced by lysine.[39] In both instances the abnormal peptide was a component of the β peptide chain. Subsequent investigations of hemoglobins E, D, and I have revealed similar amino acid substitutions in other peptides of both the α and β chains.[43] These brilliant studies have revealed that a variation of but one amino acid out of more than 300 in the half molecule can mean the difference between normal health, a moderate disability, or a fatal illness.

It has been suggested[44] that the chemical structure of the two genes which appear to be involved in the production of hemoglobin determines the amino acid sequence of the peptide chains. Any alteration of the chemical structure of the genes would alter in turn the sequence of the amino acids of the hemoglobin molecule. Since the genes are self-replicating macromolecules any structural abnormality would be transmitted from one gen

eration to the next. Whether or not the effect of such an abnormality is clinically important is believed to depend on the portion of the peptide chain involved.[43]

In addition to the protein moiety the hemoglobin molecule contains four heme groups. These groups are believed to develop coincident with[100] or slightly later than[77] the protein portion of the molecule. The four heme groups are thought to be arranged in two pairs related by the dyad axis.[40, 69] Their exact location on the molecule remains unknown.[48] On the basis of data obtained from studies of oxygen equilibrium, it has been suggested that the four hemes form a conjugated system, each heme connected to two others.[68] They are the site of the reversible binding of the respiratory gases.

Heme is a porphyrin ring structure containing a chelated iron atom and has been found to be part of the molecular structure of myoglobin, the cytochromes, peroxidase and catalase as well as hemoglobin. Porphyrins are widely distributed in nature and are found in both plants and animals where they serve many vital metabolic processes. Chlorophyll, which is essential for photosynthesis and thus for the existence of life on earth, is a magnesium porphyrin compound. A porphyrin structure with a complexed cobalt atom forms part of the vitamin B_{12} molecule.[3, 4, 91]

The Chemistry of Porphyrins

Porphyrins differ from one another and are classified according to the nature and order of the side chains which may be substituted for the eight beta hydrogen atoms. One important group of porphyrins is that which has the same two side chains substituted for the replaceable hydrogen atoms in the 3 and 4 positions in each of the constituent pyrroles. Such an arrangement

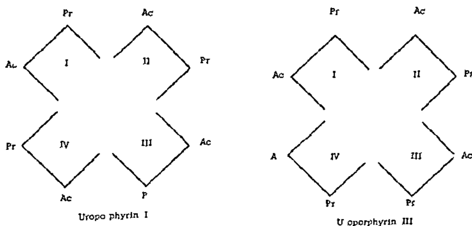


Figure 12. Schematic diagram of uroporphyrins I and III: acetic acid and propionic acid side chains.

allows four possible isomers the notations of which are I II III IV Uroporphyrin and coproporphyrin are examples of this group Only types I and III are important to man

Note that the structural difference between uroporphyrin I and uroporphyrin III lies in the position of the acetic and propionic acid side chains in pyrrole ring IV (Fig 12)

The structural difference between coproporphyrins I and III also lies in the position of the side chains located on pyrrole IV and the difference between the uroporphyrins and coproporphyrins is in the presence of a methyl group in the coproporphyrins in the position occupied by the acetic acid side chain in the uroporphyrins (Fig 13)

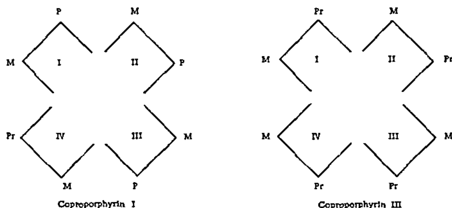


Figure 13 Schematic diagram of coproporphyrins I and III methyl and propionic acid side chains

Uroporphyrin and coproporphyrin each occur in both urine and feces despite the impression to the contrary given by their names The urine contains small amounts of uroporphyrin (5 to 10 μg/day) with both isomers present [13] Larger quantities of coproporphyrin are found in the urine (100 to 300 μg/day) and the type III isomer predominates [14, 4, 11, 6] Inadequate data are available to determine a normal range of fecal uroporphyrin excretion The quantity of fecal coproporphyrin excreted daily is from 150 to 400 μg [56, 76]

When there are three different side chains substituted for the replaceable hydrogen atoms of the constituent pyrroles a larger number of isomers are possible Mesoporphyrin is a tetramethyl diethyl dipropionic acid porphyrin and fifteen isomers have been enumerated Protoporphyrin is similar but two vinyl are substituted for the two ethyl side chains The isomer which is found in the hemoglobin molecule is classified as protoporphyrin IX type III

Porphyrin is composed of four monopyrroles The elucidation of the synthesis of porphyrin has made it clear that glycine and succinyl Co A are the basic building blocks [71, 9, 93, 94, 97, 11] Succinyl Co A from the

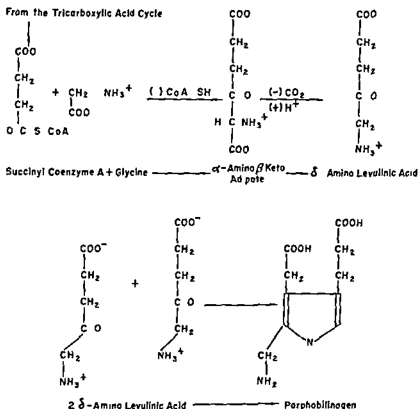


Figure 14 Formation of porphobilinogen from succinyl coenzyme A and glycine

tricarboxylic acid cycle with the addition of glycine forms α amino β keto adipic acid Pyridoxal phosphate [8] Co A [7] and ferrous iron[10] are required for the reaction as well as enzymes which appear to be localized in the mitochondria [9] α Amino β keto adipic acid is then decarboxylated to form δ aminolevulinic acid Two molecules of δ aminolevulinic acid condense to form porphobilinogen a monopyrrole which contains acetic and propionic acid side chains [8, 16] This reaction has been shown to be catalyzed by an enzyme which contains —SH groups [81] and which has been recently shown to contain copper as well [44] A dietary deficiency of copper results in a reduction of the concentration of this enzyme in the blood and liver of ducklings

A cell free extract of erythrocytes is capable of converting porphobilinogen to uroporphyrin [6, 16, 0] An enzyme porphobilinogenase has been described which is capable of catalyzing this transformation The normal result of the reaction is the production of the type III isomer but after heating the type I isomer is produced It is felt that the macrocyclic structures which are formed from porphobilinogen are porphyrinogens and that the porphyrins result from auto oxidation of these compounds [3, 7, 10] While uroporphyrin is poorly utilized by hemolysates to form copropor

phyrin uroporphyrinogen III is easily transformed to coproporphyrinogen III.[6, 78] The conversion of coproporphyrinogen III to protoporphyrin requires enzymes located in mitochondria and oxygen.[4] The biosynthesis of heme from protoporphyrin and iron is also enzyme dependent.[10]

The intermediates in the biosynthesis of heme are then uro- and coproporphyrinogens and not uro- and coproporphyrins. It appears, however, that iron is incorporated into protoporphyrin and not protoporphyrinogen.[8]

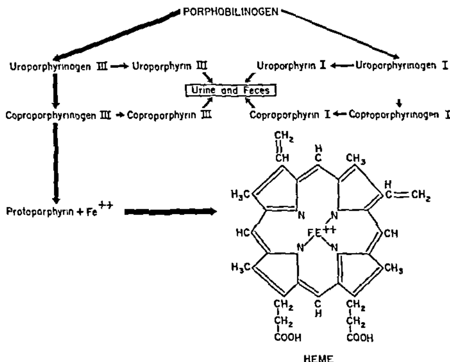


Figure 15. Formation of heme from porphobilinogen.

Free protoporphyrin and coproporphyrin have been demonstrated in both the nucleated and non-nucleated erythrocytes. The concentration of both compounds increases just prior to reticulocytosis.[10, 8, 88] It has been suggested that they represent incomplete hemoglobin synthesis[11, 5] or residuals left after hemoglobin synthesis.[64] The level of free erythrocyte protoporphyrin is from 13 to 139 micrograms/100 ml. of erythrocytes.[103] Erythrocyte coproporphyrin concentration has been found to be from 0.5 to 1.5 micrograms per 100 ml. of erythrocytes.[10, 104]

HEMOGLOBIN CATAPOLISM

Following its release from the red blood cell none of the hemoglobin is reutilized as such and each of the component parts of the molecule goes its

separate way. The protein fraction returns to the general protein pool where fragments may or may not be incorporated into future hemoglobin molecules.[83, 109, 110] Iron returns to the iron pool and is probably quickly reutilized in the formation of hemoglobin. The porphyrin fraction is not reutilized at all.

The destruction of the hemoglobin molecule begins in the reticuloendothelial cell under normal conditions. Following rupture of the methene bridge, bilirubin is formed from the heme fraction. This fragment is carried in the plasma in an insoluble form to the liver. There it is conjugated with glucuronic acid and a water soluble compound results which is excreted in the bile.[9, 80] Should the liver be unable to carry on this conjugation in the normal manner, a high "indirect acting" bilirubin level in the blood would result. Should there be obstruction to the excretion of the conjugated form via the biliary system, an increase in the "direct acting" bilirubin in the blood would result. Whether the bilirubin gives a "direct" or "indirect" van den Bergh reaction depends on the presence or absence of the glucuronic acid conjugation. The enzyme transferase found in the microsomes of the liver cells and uridine diphosphoglucuronic acid are both necessary for this reaction to occur. It has been suggested that the excess unconjugated bilirubin found in the newborn is due to inadequate development of the glucuronide conjugating system.[9]

In the bowel the action of the intestinal bacteria on bilirubin leads to several degradation products which are known collectively as fecal urobilinogen. Approximately 50 per cent of the urobilinogen is reabsorbed from the gut and recirculated in the blood to be reexcreted by the liver. This is the source of urobilinogen in liver bile and of the small amount lost through the kidney that is termed urinary urobilinogen. Normal daily excretion of urinary urobilinogen is from 0 to 3.5 mg.

The amount of fecal urobilinogen in the stool may be measured and has been found to vary from 40 to 280 mg./day. Since the amount of fecal urobilinogen excreted depends on the amount of bilirubin secreted by the liver into the bile, it has been used as an index of hemoglobin breakdown. Hemoglobin from destroyed red blood cells is not the only source of urobilinogen, however, and the excretion of fecal urobilinogen does not always measure red cell destruction accurately. Studying the excretion of stercobilin, one of the components of fecal urobilinogen, investigators have found that as much as 11 per cent must come from sources other than the breakdown of mature red blood cells.[9] Whether this represents a breakdown of developing cells while still in the marrow or some non-hemoglobin porphyrin pathway is not known. In certain disease states, the amount of non-hemoglobin stercobilin rises to as high as 50 per cent.[8, 61]

ABNORMAL PORPHYRIN METABOLISM

There are several disorders associated with abnormal porphyrin metabolism. Despite their rarity, these disorders have evoked widespread interest and have afforded unique opportunities for the study of porphyrin metabo-

lism A new approach to the investigation of protein synthesis was recently opened when it was established that δ aminolevulinic acid is a common intermediate in both porphyrin and purine synthesis [1, 9] Already the suggestion has been made that the porphyrin abnormalities found in a variety of disease states may be a reflection of abnormal purine metabolism [64] There is no doubt that continued investigation of these disorders will widen our knowledge of both protein and porphyrin metabolism

The disease states which result from disordered porphyrin metabolism may be classified as follows [83]

1 Porphyria (congenital) Erythropoietica
2 Porphyria Hepatica
   a Intermittent acute
   b Cutanea tarda
   c Mixed

1 *Porphyria Erythropoietica*

Porphyria erythropoietica is a rare metabolic disorder of the erythrocyte which is probably transmitted as a recessive genetic abnormality [101] Patients with this disorder appear to have both normal and abnormal erythroid cell lines *in the marrow* [83] *The nuclei and to a lesser extent the cytoplasm of cells* of the affected line show a characteristic red fluorescence when studied with the fluorescence microscope In addition they may have a dark nuclear inclusion body containing hemoglobin As destruction of circulating erythrocytes cannot account for the quantity of porphyrin excreted by these patients it has been postulated that uroporphyrin may be released into the plasma at a rapid rate during the maturation of the nucleated red cells in the marrow [8] The red fluorescence which has been demonstrated in the spleen is thought to result from the local destruction of red blood cells containing the abnormal porphyrin The isomers found in the urine and blood of these patients are copro and uroporphyrin I These porphyrins are not complexed with a metal and the monopyrrole porphobilinogen is not found

Recent work[77] suggests that the metabolic error of the abnormal erythroid *precursors in the marrow is occasioned by the genetically induced loss* or alteration of an enzyme essential for the formation of uroporphyrin III from porphobilinogen The alternate formation of excessive quantities of uroporphyrin I which cannot be used in heme synthesis is believed responsible for the clinical manifestations of the disorder [3, 6, 63, 11]

The clinical picture is characterized by photosensitivity which leads to erythema blisters ulcer formation and ultimate mutilation of exposed areas Hemolytic anemia is usually present and splenomegaly is expected Because of the deposition of uroporphyrin I in the bones and teeth these structures are often red brown and will fluoresce under a Wood s light [101]

Treatment is non specific Sunlight must be avoided Splenectomy has been demonstrated[43] to relieve the hemolytic anemia in a number of patients

2 *Porphyria Hepatica*

The site of deranged porphyrin metabolism in this disorder is not the bone marrow but the liver[83] Porphyria hepatica may be the result of a metabolic defect in the synthesis of heme proteins other than hemoglobin Catalase synthesis has for example been shown to be abnormal in experimental porphyria[84] These disorders are more common than the erythropoietic type

a Intermittent Acute Type The intermittent acute type of porphyria hepatica is a familial disorder inherited as a autosomal dominant It usually appears after puberty though in rare cases it may be evident in childhood[101] In this disorder the erythrocyte porphyrins are normal but excessive quantities of zinc complexed uroporphyrin I and III and coproporphyrin III are found in the urine The porphyrin precursor porphobilinogen, is found in the urine during the acute attacks and the finding of this monopyrrole is diagnostic The liver contains large quantities of porphyrin precursors and increased amounts of porphyrin Marrow porphyrins are normal

The clinical manifestations of the intermittent acute type of porphyria hepatica are varied but abdominal colic—which can be of extreme severity—weakness and paresthesia of extremities constipation convulsions coma psychoses and hypertension may occur Fatalities are generally due to respiratory paralysis but may occur during coma or from cachexia[101]

Treatment includes avoidance of alcohol barbiturates or other liver toxins Recently[107] chlorpromazine has been reported effective in relief of symptoms and this has been confirmed[101] Splenectomy is not indicated

b Cutanea Tarda Type As its name suggests this disorder begins later in life and its symptoms include photosensitivity It is a familial disorder associated with parenchymal liver disease in a high percentage of cases The photosensitivity is moderate in severity The disease runs a mild course if the patients protect themselves from exposure to sunlight and hepatotoxins A history of chronic alcoholism is not uncommon The porphyrins excreted are the zinc complexed uroporphyrin I and III and coproporphyrin III Porphyrins may be found in the blood and are elevated in the liver Porphobilinogen is not found in the urine of these patients The prognosis is generally good but depends on the degree of liver damage

c. Mixed Type There is considerable disagreement[107] regarding this designation It includes those patients with cutanea tarda who develop in addition symptoms of the acute intermittent variety The urine contains uroporphyrin I a type III porphyrin and porphobilinogen as well

Porphyrinurias

There are a number of conditions in which increased porphyrin excretion may occur The increased excretion of porphyrin in these varied disease states may reflect disordered hemoglobin or protein synthesis or disordered synthesis of heme compounds other than hemoglobin

1 Lead Poisoning—coproporphyrin III uroporphyrin (Type III?) porphobilinogen[4]
2 Rheumatic fever—coproporphyrin III[47]
3 Poliomyelitis—coproporphyrin III[109]
4 Liver disease[108]
   a Alcoholic cirrhosis—coproporphyrin III
   b Infectious hepatitis—coproporphyrin I
5 Anemia[83, 10, 106]
   a Refractory—coproporphyrin III
   b Hemolytic anemia—coproporphyrin I
   c Pernicious anemia—coproporphyrin I
6 Idiopathic—coproporphyrin III[106]

IRON METABOLISM

Iron is an essential element in many of the physiological processes in the body It combines with protoporphyrin and various proteins to form hemoglobin and many important enzymes such as catalase cytochrome and peroxidase Since iron plays an essential role in hemoglobin metabolism some knowledge of iron metabolism is necessary for the understanding and proper management of many of the problems of anemia seen in clinical practice The history of iron in medicine has been reviewed by Fowler[1, 3] and excellent summaries of the current views on iron metabolism have been published by Moore and Dubach[134] Gubler[1, 7] and Demulder[110]

SOURCES OF IRON

The average daily diet of healthy adults in the United States contains 12 to 15 mg of iron which is mainly in the ferric state[136] As the normal adult absorbs less than 10 per cent of this amount the quantity of iron absorbed each day is in the range of 0 6 to 1 5 mg[136] Good dietary sources of iron are liver beef muscle eggs molasses dried fruits and spinach Bread fortified with iron is another effective source[136]

ABSORPTION OF IRON

Iron in the lumen of the gastrointestinal tract is still outside the body in a physiological sense and the factors that influence its absorption are probably more important than the quantity of iron that is ingested In 1933 Lintzel demonstrated that iron is absorbed from the gastrointestinal tract in the

ferrous form[1] When 0 05 to 0 20 per cent of alpha alpha dipyridyl a compound that reacts specifically with ferrous iron to form a complex that is red in color was added to the diet of young rats it was observed that binding of the ferrous iron occurred in the pyloric area at the pH of the stomach The iron that was bound in this manner was excreted in the feces The growing rats which were deprived of ferrous iron by the alpha alpha dipyridyl binding became anemic and it was concluded that no significant amount of iron was absorbed in the ferric state Other investigators have concluded that in man iron is absorbed from the gastrointestinal tract almost entirely in the ferrous form[137 148] The conversion of the ferric iron of the food to the available ferrous forms occurs mainly in the stomach or upper small intestine Certain proteins and ascorbic acid promote iron absorption because they are capable of reducing ferric iron to the ferrous state at an acid reaction other compounds such as phosphatides and phosphoproteins hinder iron absorption[148]

The quantity of iron in the body is regulated by the amount of iron that is absorbed There is no balanced mechanism of absorption and excretion like that which exists for many metals such as calcium and sodium This was first shown in 1937 by Widdowson and McCance who studied the *urinary and fecal excretion of iron by two men and two women on a low* iron intake on a very high iron intake of 1000 mg a day and when they were again placed on a low iron intake[1 0] It was observed that on the low iron intake practically all of the ingested iron was recovered in the feces and the subjects were considered to be "in balance During the high iron intake period when each subject ingested 1000 mg per day for 36 to 40 days it was calculated that each individual absorbed from 2000 to 5000 mg of iron without any significant increase in the hemoglobin levels When the subjects were again placed on a low iron intake of 7 to 10 mg a day for a period of eight to ten days they excreted all of the iron ingested during that time but none of the iron that was absorbed during the preceding period of high iron intake From these studies it was concluded that the intestine has no power to regulate iron by varying the amount excreted in the feces and therefore the amount of iron in the body must be regulated by the amount that is absorbed

A similar conclusion regarding the important role of the mucosa of the gastrointestinal tract in iron absorption was reached in 1943 by Hahn Bale Ross Balfour and Whipple who utilized radioactive iron to study iron metabolism in dogs[1 8] The effects of various factors on iron absorption were studied The amount of the radioactively tagged iron that appeared in the red cells was considered to be a measure of iron absorption They found that when acute anemia was produced in the dogs by bleeding there was no increase in the absorption of iron until more than seven days had elapsed In contrast chronically anemic dogs promptly absorbed iron at from ten to twenty times the normal rate Another experiment suggested that the lowered oxygen content of anemic blood was not the factor responsible for increased absorption of iron in anemia anoxia produced by breathing a gas

that contained only 50 per cent of the normal concentration of oxygen for 48 hours did not have any significant effect on the absorption of iron A lack of control of absorption of iron by the state of the iron stores was suggested by the observation that administration of colloidal iron intravenously in an amount sufficient to saturate the body stores did not block the iron absorption from the gastrointestinal tract three days later Colloidal iron given orally acted differently Iron administered orally produced a partial block to the absorption of radioactive iron when the latter was administered orally from one to six hours later From these studies it was concluded that "physiological saturation" with iron is a term that could be applied to the mucosal epithelium of the gastrointestinal tract to explain one phase of the acceptance or refusal of iron In these experiments desaturation" appeared to be a matter of days while "saturation" was a matter of one or two hours Although these experiments of Hahn et al are important certain details of the methods restrict the conclusions that can be drawn All of the experiments were done with metallic iron not food iron The amount of radioactive iron that appeared in the erythrocytes was used as a measure of iron absorption but it is really a measure of both iron absorption and erythropoiesis The conclusions regarding the effect of anemia on iron absorption were drawn from test animals that were deficient in iron

The Mucosal Block Theory

In order to explain the mechanism whereby the gastrointestinal tract mucosa regulates iron absorption Granick has proposed the mucosal block" theory of the regulation of absorption of iron by ferritin in the mucosal cells[1 6] Ferritin is a compound of iron and a protein apoferritin Iron which occurs on the surface of this compound as small micellar units of ferric hydroxide can make up as much as 23 per cent by dry weight of this compound Ferritin was identified in the duodenal mucosa of guinea pigs and it was observed that variations in the quantity of ferritin present in the mucosa paralleled the increase or decrease of resistance to iron absorption It was also found that the amount of apoferritin in the mucosal cells paralleled the amount of ferritin an observation which suggested that the absorption of iron stimulated the formation of the specific protein, apoferritin These observations form the basis of Granick's theory that ferrous iron from the lumen of the gastrointestinal tract enters the mucosal cells until all the apoferritin is converted to ferritin The ferrous iron is then considered to be in equilibrium with the ferric iron of ferritin In this circumstance a state of physiological saturation with the ferrous iron exists and no more iron is absorbed Only when iron has passed into the plasma does the ferric iron of the mucosal ferritin decrease When this occurs physiological saturation" no longer exists and more ferrous iron can be absorbed The outline of this theory is shown in the accompanying diagram (Figure 16)

The ferritin mucosal block theory appears to be the best explanation for many of the details of iron absorption that have been demonstrated but not

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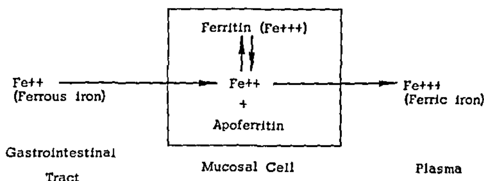


Figure 16 Schematic representation of the mucosal block theory (after Granick)

all of the observations are explained by this theory Dubach Callender and Moore who utilized radioactive iron to study iron absorption as well as iron utilization found that the level of plasma iron and the presence of anemia per se did not influence iron absorption they were unable to demonstrate the presence of any iron absorption promoting factor" by the injection of *plasma of patients with iron deficiency*[1 0] *In addition Pirzio-Biroli Both* well and Finch have shown in both experimental animals and human subjects that large iron stores cause a diminution in the absorption of iron while increased erythropoiesis is associated with an increase in absorption[139] In these studies individuals who were apparently normal showed considerable variation in absorption that could not be explained

In idiopathic hemochromatosis there appears to be a breakdown in the regulation of iron absorption and as a result there is an increase in the absorption of iron which leads to a great increase in the stores Several observations indicate that the control of iron absorption is only partial or even ineffective at times in other conditions as well as in idiopathic hemochromatosis In patients with idiopathic hemochromatosis and large iron stores iron absorption increases when erythropoiesis increases following phlebotomy[117] It has been demonstrated also that iron will be absorbed in spite of adequate stores by normal individuals and by patients with refractory anemia pernicious anemia in relapse and hemolytic anemia even though the iron is not used for hemoglobin production[11, 1 0]

The lack of absolute control of absorption by the mucosal cells when faced with a large amount of iron in the lumen of the gastrointestinal tract was also shown by Whipple and Robscheit Robbins[140] It was observed that whereas the percentage of iron absorbed was high with small doses of iron the actual amount used in hemoglobin production was larger when large doses of iron were administered even though the percentage of utilization was smaller A similar observation was reported by Hegsted Finch and Kinney who found that iron increased in the liver in animals on a normal diet when iron was added[130] *They concluded that the normal regulatory* mechanism is effective only when the iron phosphate ratio of the diet is

within normal limits and at low levels of iron intake since both the absolute amounts of iron and phosphate and the iron phosphate ratio in the diet influence the amount of iron that is absorbed The studies that have been reported on factors influencing iron absorption were the subject of a recent critical review [134]

Absorption of Iron in Food

Most of the studies on iron metabolism have been concerned with the action of inorganic iron salts Moore and Dubach have investigated the important problem of the absorption of iron contained in foodstuffs [135] They studied the effectiveness of bread fortified with radioactive iron spinach grown in nutrient solution containing radioactive iron as well as egg liver and muscle from hens that had been injected with radioactive iron Normal individuals rarely absorbed more than 10 per cent of the iron in the food Iron deficient patients absorbed some types of food iron better than normal individuals but this increase in absorption did not occur with iron incorporated in eggs Bread fortified with various iron preparations was effective since normal individuals absorbed 1 to 2 per cent of the iron while three patients with iron deficiency anemia absorbed 45 to 64 per cent The effect of other foodstuffs on the absorption of the iron containing foods was tested and only ascorbic acid was found to produce a marked increase in iron absorption It is interesting and somewhat surprising that no increase in the absorption of iron could be demonstrated after the addition of dilute hydrochloric acid to the diet of patients with hypochlorhydria

Although the patient with iron deficiency anemia absorbs more iron from the normal diet than the normal individual does [117 138 139] dietary iron may be inadequate when the anemia is severe It is very difficult for even the severely anemic patient to absorb more than 5 mg of iron from a normal diet [1] and usually the amount of food iron absorbed by an iron deficient patient is considerably less than 5 mg per day In such circumstances supplementary medicinal iron is generally necessary to correct the deficiency in stored iron [117] The absorption of food iron is less than normal even though anemia is present in patients with idiopathic steatorrhea infection and myxedema [139] After partial gastrectomy the ability to absorb food iron is lost or seriously diminished but the ability to absorb oral iron salts is increased as in any patients with iron deficiency [117 139]

TRANSPORT OF IRON

On leaving the mucosal cells iron is transported in the plasma in the ferric form attached to a beta 1 globulin fraction of protein variously termed transferrin siderophilin iron binding protein or metal binding globulin This binding component of the serum was reported in 1945 by Holmberg and Laurell who utilized alpha alpha dipyridyl to demonstrate that normal serum could contain only about 300 micrograms per 100 ml of iron in a firm binding [132] They concluded that the toxic effects of intravenously ad

ministered iron which had been observed by Waldenstrom occurred when the saturation limit of the plasma was exceeded and the surplus iron left the blood stream. At about the same time in 1946 Schade and Caroline reported the presence in human plasma of a protein fraction that possessed the capacity to bind iron at physiological pH and identified it as being in the beta 1 globulin fraction.[144] Each molecule of this protein combines with two molecules of ferric iron.[1, 7, 136] The protective effect of the protein binding of the iron in the blood has been demonstrated by means of intravenous injections of iron preparations. Poorly dissociated iron compounds such as iron saccharide can be given in large amounts intravenously whereas the easily dissociated iron compounds can be given only in small amounts of 5 to 10 mg. Larger amounts of easily dissociated iron compounds quickly saturate the total iron binding capacity of the serum and the excess iron causes flushing, nausea, vomiting, shock and even death.

The normal plasma iron level in micrograms per 100 ml has been reported to be 50 to 150,[136] 80 to 160[113] and 43 to 210.[11] In the latter study the mean was 104.7 ± 3.4. In some series the plasma iron values for males have been higher than those for females; in others there has been no significant difference. In one series the mean serum iron for men was 119.6 ± 6.7 micrograms per 100 ml (range 61-228) and for women was 101.9 ± 5.5 micrograms per 100 ml (range 35-246);[143] in another the ranges were 81 to 162 micrograms per 100 ml in men and 64 to 128 micrograms per 100 ml in women.[131] Large unsystematic daily variations in the serum iron levels that amounted to 100 per cent were noted in some patients in one series.[143] The serum iron level is usually highest in the morning and falls in the evening. In one study mean changes were −35.3 ± 6.0 for men and −16.1 ± 3.6 micrograms per 100 ml for women.[143] A mean serum level of 107.6 micrograms per 100 ml has been reported in a group of boys and girls four and a half to eight years of age.[143] In children two to four and a half years of age the mean for boys was 95.9 micrograms per 100 ml and for girls 59.7 micrograms per 100 ml.[143]

Plasma iron, which has a complete turnover every two or three hours,[114, 133] is the iron that is being transported to the storage areas and to the marrow for hemoglobin production and also iron that may be destined for other use. Studies with radioactive iron have indicated that the plasma iron turnover rate is 0.56 mg per kilo per day and the red cell iron turnover rate is 0.52 mg per kilo per day in an adult male.[114] In a 70 kilo man this represents a plasma iron turnover of 39 mg per day with 36 mg being used for hemoglobin production. Others have found the plasma turnover rate to be 37.5 mg a day and the iron utilization for hemoglobin production to be 29 mg per day. These values exceed the theoretical figure of 18.5 to 22.3 mg per day for hemoglobin production.[1, 4] From these studies it was concluded that measurements of plasma iron utilization do not measure effective erythropoiesis, and it was suggested that the excess turnover in the plasma may represent exchange of iron with other tissues or the production in the marrow of hemoglobin which does not enter the circulation in the form of viable erythrocytes.[124]

The studies of Jandl and others indicate that plasma iron which is firmly bound to human iron binding protein is transferred preferentially to immature but not mature erythrocytes This transfer which is related to metabolic energy producing processes is dependent upon the extent of saturation of the iron binding protein and is maximum in the normal range of 20 to 60 per cent saturation [133]

The total iron binding capacity of normal serum has been reported to be in the range of 300 to 359[115] micrograms per 100 ml or as high as 420 [136] Normally the protein is about 33 per cent saturated [110]

Plasma iron levels and the amount of iron binding protein in the plasma vary in certain disease states The serum iron level is increased in hemolytic anemia pernicious anemia aplastic anemia, hemochromatosis and hemosiderosis [11] Under such circumstances the total iron binding capacity of the serum is not increased The serum iron level is increased in infectious hepatitis and the rise appears to be correlated with the degree of parenchymal necrosis [14] [147] In iron deficiency anemia the plasma iron is decreased while the total iron binding capacity is usually greatly elevated Similar changes usually occur during the latter part of pregnancy In infections both the plasma iron and total iron binding capacity are usually lower than normal A decrease in the plasma iron may be noted within 24 hours after the production of fever [11] [116] A rise in the plasma iron may be noted in less than an hour after iron is given orally (Values for the serum iron and latent iron binding capacity observed in various disease states are shown in Table 2)

Table 2 Actual Values of Serum Iron and Latent Iron Binding Capacity Observed in Patients with Various Disorders

Diagno	Hb (gm %)	RBC (m ll / bi millim t)	Hct (%)	SI (γ%)	LIBC (γ%)
N m l	15 0	5 0	46	105	200
I D fi l y	5 0	3 0	21	12	650
I D fi l y m d t ly	9 4	4 0	33	17	585
I D fi l y mild	12 4	4 0	40	47	476
Apla ti An mi idiop th (unt t d)	6 2	1 7	18	137	270
Apla ti A mi d t d g (?) (1 t d)	5 9	1 4	14	260	Sat
Apla ti A mi id op thi (397 t f l)	6 5	1 5	18	142	Sat
Id op thi H m h m to i	14 0	4 8	43	105	S t
Vi l H p titi	14 2	4 5	46	212	Sat
Inf tio	14 5	4 7	44	28	268

STORAGE OF IRON

Iron is stored in the fixed phagocytic tissues of the reticuloendothelial system of the liver, spleen and bone marrow and also in the parenchymal cells of the liver and glandular tissues such as the pancreas and adrenals, mainly in the form of ferritin and hemosiderin.[1] Ferritin contains up to 23 per cent of iron in the ferric form and hemosiderin, also a compound of ferric hydroxide and protein, contains from 9 to 55 per cent. Studies of patients with excess iron stores who have been subjected to repeated bleedings have demonstrated that iron from both these compounds can be used for hemoglobin formation. The relationship of these compounds to each other is still not certain, but some authors state that hemosiderin and ferritin are intimately associated and functionally indistinguishable and consider it likely that the compounds differ only in physical form.[11]

Finch et al. in 1950 studied the iron absorption in dogs by determining the distribution of radioactive iron given orally and intravenously.[1] They found equal amounts of radioactivity per gram of tissue in the liver and spleen after administering radioactive iron in the food and a similar distribution as soon as a few hours and as long as twenty-one days after intravenous injections. They observed that small or large amounts of iron were distributed similarly, but most of the iron gaining entrance to the body by either the oral or intravenous route was stored in the liver because of its size. In hemolytic anemias and after blood transfusions most of the iron is found in the spleen, probably because this organ is the main site of erythrocyte destruction.

The total amount of iron in the body in adults is between 3.0 and 5.0 grams.[118, 119, 127, 129, 136] Some authors state that about 73 per cent of this iron is contained in the hemoglobin,[118, 127] while others give figures of 55 per cent[136] and 1.5 to 3.0 gm.[129] The amount of iron contained in the body in the form of myoglobin, catalase and cytochrome has been estimated as 100 to 300 mg[118] or 3.5 per cent.[127] The remainder of the iron is storage iron, which constitutes from 16 to 23 per cent of the total, about 1.0 to 1.5 grams.[118, 129, 136]

Table 3. Iron Values in the Human Adult According to Various Authors[118, 119, 127, 129, 136]

Daily dietary intake	12–15 mg
Daily absorption	0.6–1.5 mg
Total body iron	3.0–5.0 gm
Hemoglobin iron	1.5–3.0 gm
Storage iron	1.0–1.5 gm
Parenchymatous iron	0.1–0.3 gm
Daily loss, males	0.5–1.5 mg
Daily loss, females	1.0–2.5 mg
Daily loss, pregnant females	1.0 mg

Either a deficiency or an excess of storage iron may be important clinically. In severe iron deficiency the iron stores are largely depleted and are corrected only with difficulty by the administration of oral iron, since the increased absorption of iron that exists when iron deficiency anemia is present seems directed toward hemoglobin production and absorption decreases markedly when the hemoglobin level is restored to normal.[1] For this reason extra iron must either be given orally for long periods or administered parenterally in order to restore the iron reserves.

Iron excess occurs in the form of hemosiderosis or hemochromatosis. In the former abnormally large amounts of iron are deposited in the reticuloendothelial cells of the spleen, liver and bone marrow. This situation, which is not associated with serious physiologic derangement, is seen chiefly in hemolytic anemia and after numerous blood transfusions. In "idiopathic" hemochromatosis, which results from increased iron absorption from the gastrointestinal tract, there is an excess of tissue iron associated with fibrosis of the liver and pancreas and also increased iron in the parenchymal cells of the liver. Clinical features such as portal cirrhosis, cardiac failure, pigmentation of the skin and diabetes mellitus often occur. Finch and Finch have published a comprehensive review of their work and the literature on the subject of iron storage disease.[1 1] Whether or not iron excess per se, either as a result of increased absorption from the gastrointestinal tract or as a result of numerous transfusions, can produce tissue damage that occurs in both "idiopathic" hemochromatosis and "exogenous" hemochromatosis remains an unsettled point.[1 1 141]

EXCRETION OF IRON

The amount of iron in the body is determined largely by regulation of absorption. The body seems to conserve iron in a tenacious fashion and a very small amount is lost daily. This occurs by desquamation of cells of the skin and gastrointestinal tract, by the migration of leukocytes into the gastrointestinal tract, by the shedding of hair and by excretion of bile, urine and sweat. Moore and Dubach found the fecal excretion of normal persons to be 0.3 to 0.5 mg. per day, whereas in iron deficiency the amount may be only one tenth as much.[136] Thus the average adult male loses about 0.5 to 1.5 mg. per day and the female loses an average of 1.0 to 2.5 mg. per day because of menstruation. During pregnancy the female loses an additional 1 mg. per day since the fetus is furnished a total of 0.3 to 0.5 gram and other iron is lost at delivery. The normal excretion of only 1.0 mg. per day indicates that it would require many years for a deficient diet to produce iron deficiency anemia in an adult male; for this reason, therefore, iron deficiency in the adult male should be considered primarily as a sign of chronic blood loss. Whereas the conservation of iron is generally advantageous to the body, the inability of the body to excrete more than around 1.0 mg. a day is a factor that promotes the increase of body iron when excessive amounts are administered by any route. At the

present time bleeding by phlebotomy or by some other means, is the only known way to reduce the iron stores.

SUMMARY

The metabolic pathways of iron are shown schematically in Figure 17. The daily diet of the average adult in the United States contains 12 to 15 mg of iron which is present mainly in the ferric form. Reduction to the ferrous form, the type of iron that is absorbed, is promoted by the acid reac-

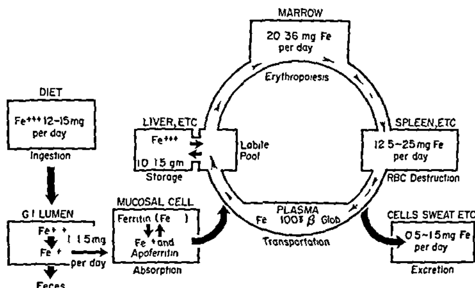


Figure 17 Schematic representation of the metabolic pathways of iron

tion of the stomach and upper small intestine by ascorbic acid and by certain proteins. Iron is absorbed mainly in the upper portion of the small intestine and the amount of iron that gains entrance to the body is largely controlled by regulation of the absorption of iron through the mucosa. Normally less than 10 per cent of the ingested iron is absorbed. Various factors such as the amount and form of iron in the intestine, the iron phosphate ratio and possibly other details of the diet, the status of the iron stores, the degree of erythropoietic activity and perhaps other factors influence this absorptive mechanism. After absorption the iron is transported in the plasma in the ferric state in combination with a beta 1 globulin fraction to the reticulo-endothelial cells of the liver, spleen and bone marrow where it is stored in the form of ferritin and hemosiderin. From these stores the iron is mobilized for hemoglobin production in the bone marrow. The body has no means of excreting significant amounts of iron and only minute amounts of iron about

1 mg per day are lost by cell desquamation and other means Reduction of the amount of iron in the body occurs mainly as the result of bleeding and pregnancy

ADDITIONAL FACTORS NEEDED FOR ERYTHROPOIESIS

In addition to an adequate balance of iron and protein other metals and certain vitamins must be present if normal erythropoiesis is to continue[1]

Copper Although anemia due to copper deficiency has not been produced in adult humans in experimental animals a deficiency of copper leads to the development of an anemia that is similar to iron deficiency anemia The adult human body contains 100 to 150 mg of copper[11] The plasma level has been found to be 105 ± 16 micrograms per cent in males and 116 ± 16 micrograms per cent in females Ninety per cent of the copper in the plasma is bound to the alpha 2 globulin ceruloplasmin[39] Copper appears to function in the absorption mobilization and utilization of iron in hemoglobin synthesis and in the formation and maintenance of the cytochromes[86, 11] The metal may exert an effect on hematopoiesis through an effect on the function of respiratory enzymes Recently copper has been shown to be a component of an enzyme essential for the synthesis of porphobilinogen from δ aminolevulinic acid[44] thus establishing its role in heme synthesis

Cobalt Cobalt is known to play a role in animal nutrition and the administration of cobalt is known to stimulate erythropoiesis in man and in animals[66] However the mechanism by which cobalt effects an increase in red blood cell production is not understood[54] It has not been established that cobalt has any role in normal erythropoiesis aside from its importance as a part of the vitamin B_{12} molecule

Pyridoxine For many years it was known that a deficiency of pyridoxine (vitamin B_6) in animals would produce a severe hypochromic microcytic anemia but only recently has a similar disorder been described in man[3] The deficiency which is corrected by the administration of pyridoxine is characterized by splenomegaly hepatomegaly hypochromic microcytic anemia elevation of serum iron level and saturation of the iron binding capacity The inability of these patients to metabolize iron normally is associated with disturbed tryptophan metabolism and the excretion of xanthurenic acid.

Vitamin B_{12} and Folic Acid Both vitamin B_{12} and folic acid are necessary for the normal maturation of the red cells A deficiency of either

results in a defect in nucleic acid metabolism that is reflected by the development of the megaloblastic type of erythrocyte maturation Vitamin B_{12} has now been identified as the *extrinsic factor* of Castle An *intrinsic factor* in the stomach a *mucoprotein is necessary in order that the vitamin B_{12}* ingested in the diet be absorbed [13] The chemical characteristics of the intrinsic factor have been worked out in considerable detail[31, 98, 111] An absence or deficiency of the intrinsic factor is responsible for pernicious anemia The mode of action of the intrinsic factor is not known though it is suspected that it acts to catalyze the absorption of vitamin B_{12} [111] Folic acid also present in the diet does not require the intrinsic factor for its absorption The functions of these two vitamins are not completely understood Both are concerned with the formation of purine or pyrimidine bases which are constituent parts of the nucleic acids There seems to be a reciprocal relationship between vitamin B_{12} and folic acid in the synthesis of nucleic acid in many tissues of the body particularly those with rapid turnover such as the hematopoietic and gastrointestinal cells[3, 6]

Ascorbic Acid Ascorbic acid appears to participate in metabolic activities which affect both vitamin B_{12} and folic acid It is important in the conversion of folic acid to folinic acid and there is some evidence that it stimulates the formation of folic acid This vitamin has intense reducing properties and must play a significant role in oxidation reduction reactions in the body [6, 36]

Other vitamins may possibly play a role in erythropoiesis Nicotinic acid is thought to be essential for the synthesis of coenzymes in the developing erythrocyte but its role in hematopoiesis is not clearly established[34] Riboflavin deficiency has been reported to be responsible for anemia in animals but no anemia of this type has been recognized in man It is thought to exert its influence by taking part in cellular oxidations[99]

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CHAPTER III

Mechanisms and Diagnosis of Anemia

THE CLASSIFICATION OF ANEMIA

PROBLEMS related to anemia constitute a large segment of investigative hematology and an appreciable part of any clinical practice The present chapter is devoted to the classification of anemia and to brief discussions of the underlying mechanisms that are involved in each type of anemia The different mechanisms are discussed at this time to emphasize the importance of these fundamentals and to provide a basis for the development of a unifying systematic approach to the various clinical syndromes More detailed discussions of the pathophysiology of most of the individual syndromes that are associated with anemia are given in Chapter IV

Anemia may be defined as a reduction in the circulation of either hemoglobin or erythrocytes It occurs whenever the hematopoietic equilibrium is disturbed and the loss of erythrocytes or hemoglobin from the circulation exceeds production

Anemia is usually classified according to either causative factors or mor

phologic characteristics Both classifications are important because neither is entirely satisfactory Classification according to causative factors may be somewhat ambiguous because various details of a disease may be considered as causes Thus anemia may be caused by chronic blood loss the blood loss may be due to several causes" such as excessive vaginal bleeding and hemorrhoidal bleeding Each of these types of bleeding may arise from one of several causes such as functional menorrhagia or uterine malignancy in one circumstance and simple hemorrhoids or hemorrhoids associated with cirrhosis of the liver in the other Difficulty in classification also arises if the mechanism is viewed as a "cause when anemia results from more than one factor such as increased destruction and diminished production of erythrocytes Classification according to the morphologic characteristics of the erythrocytes is not entirely satisfactory either because the same underlying disease may produce more than one type of anemia "Aplastic anemia may be normocytic or macrocytic chronic bleeding may produce a simple microcytic type of anemia at one stage and a hypochromic microcytic type of anemia at another Although each classification has shortcomings when used alone both are very helpful in clinical hematology when they are used together Determinations of the morphologic characteristics of the anemia and of the mechanism of its production are both important steps toward the identification of the underlying cause which is the objective of diagnosis

Morphologic Classification

The morphologic classification of anemia is helpful in diagnosis because the characterization of the anemia according to the size and hemoglobin content of the erythrocytes directs the future investigation toward a definite group of possible causative factors or clinical syndromes and eliminates others from consideration In order to classify anemia morphologically it is necessary to determine the size and hemoglobin content of the erythrocytes With the proper experience abnormalities in these details can be detected when a well prepared smear of the peripheral blood is examined carefully In other circumstances the size and hemoglobin content of the erythrocytes can be determined most satisfactorily by the formulae devised by Wintrobe [87]

Mean Corpuscular Volume (MCV) (in cubic microns c μ)	=	Vol packed red cells (in cc per 1000 cc blood) / R B C (in mil per cu mm)
Mean Corpuscular Hemoglobin (MCH) (in micromicrogram γγ)	=	Hb (in gm per 1000 cc blood) / R B C (in mil per cu mm)
Mean Corpuscular Hemoglobin Conc (MCHC) (in per cent %)	=	Hb (in gm per 100 cc) × 100 / Vol packed R B C (in cc per 100 cc blood)

Wintrobe's classification of anemia according to cell size and hemoglobin content is given in Table 4 with slight modifications

Table 4 Classification of Anemia According to Size and Hemoglobin Content of the Erythrocytes[5]

TYPE	MCV (c µ)	MCHC (%)
1 Macrocytic	> 94	> 30
2 Normocytic	80-94	> 30
3 Simple Microcytic	< 80	> 30
4 Hypochromic Microcytic	< 80	< 30

Classification According to Mechanism

If "cause" of the anemia is thought of in the restricted sense of the immediate cause or "mechanism," a workable classification of anemia can be devised Such a classification helps the physician focus on this aspect of the problem and provides a sound basis for further systematic investigation or treatment A classification of anemia according to causative mechanisms is presented in Table 5

Table 5 Classification of Anemia According to Mechanism of Production

MECHANISM	CAUSE	DISEASE OR CLINICAL SYNDROME
I DIMINISHED ERYTHROPOIESIS		
A. Nutritional Deficiency		
1 Diet	Inadequate intake	Multiple deficiencies
2 Defective absorption Stomach	Failure to secrete intrinsic factor	Vitamin B_{12} deficiency (Pernicious anemia)
	Total gastrectomy	
	Partial gastrectomy	Iron deficiency
Intestine	Diarrhea Diverticula Fistulas Stricture	Folic acid, vitamin B_{12} or iron deficiency (Sprue malabsorption syndrome or pernicious anemia)
3 Increased demand	Pregnancy	(Folic acid, vitamin B_{12} or iron deficiency)
	Growth	Iron deficiency
B Bone Marrow Failure	1 Associated Disease	Primary disease
	2 Drugs	Aplastic anemia
	3 Chemicals	Aplastic anemia
	4 Irradiation	Aplastic anemia
	5 Endocrine	Myxedema, pituitary disease
	6 *Idiopathic*	Aplastic anemia
II BLOOD LOSS		
A Acute	Trauma or disease	Shock or anemia
B Chronic	Lesion of gastrointestinal tract or gynecologic disturbance	Iron deficiency anemia or *primary disease*

Table 5—(Continued)

MECHANISM	CAUSE	DISEASE OR CLINICAL SYNDROME
III INCREASED HEMOLYSIS		
A Intracorpuscular	Congenital defects	Hereditary spherocytosis Sickle cell and other hemoglobinopathies Thalassemia Drug sensitivity Paroxysmal nocturnal hemoglobinuria Congenital non spherocytic anemia
B Extracorpuscular	Acquired Defects	
	1 Iso antibodies	Erythroblastosis fetalis transfusion reaction
	2 Auto antibodies	Idiopathic and secondary auto immune hemolytic disease
	3 Auto hemolysis	Paroxysmal cold hemoglobinuria
	4 Infections	Malaria septicemia etc
	5 Chemicals	Phenylhydrazine etc
	6 Physical agents	Burns
	7 Vegetable poisons	Favism (also intracorpuscular)
	8 Miscellaneous	Splenomegaly liver disease hemopathic hemolytic anemia

MECHANISMS RESPONSIBLE FOR ANEMIA

I DIMINISHED ERYTHROPOIESIS

When anemia results from diminished blood production study of the peripheral blood usually reveals a normocytic or macrocytic anemia associated with a low or normal level of the reticulocytes Leukopenia and thrombocytopenia are often present If the diminished blood production is the result of vitamin B_{12} or folic acid deficiency erythropoiesis is megaloblastic in type if the anemia is due to other causes erythropoiesis is normoblastic Blood production may be inadequate because there is (a) a deficiency of the factors that are essential for erythropoiesis or (b) a failure of the bone marrow to utilize the essential substances although they are available

A Nutritional Deficiency

The factors essential for normal erythropoiesis are metals (cobalt iron copper) protein (certain amino acids) and vitamins (vitamin B_{12} folic acid ascorbic acid pyridoxine niacin and possibly riboflavin pantothenic acid and thiamine)[89] A deficiency of any one of these factors might be expected to produce anemia A lack of amino acids appears to be an unlikely

cause of anemia in man since amounts of these substances sufficient for erythropoiesis become available from some body source even when the serum protein levels and the protein intake are exceedingly low [13] In man only deficiencies of folic acid vitamin B_1 iron, and ascorbic acid are important in the production of anemia [8] A deficiency of these essential factors may arise in various ways such as (1) faulty diet (2) defective absorption or (3) excessive demands for erythropoiesis

1 *Faulty diet* is responsible for anemia in various parts of the world in usual or unusual circumstances In the United States except in food faddists or alcoholics nutritional deficiency is rarely a cause of anemia unless other factors operate to produce a conditioned or secondary deficiency [89] When anemia occurs in adults because of faulty diet the anemia is usually *the result of folic acid deficiency* possibly because of the large body reserves of vitamin B_{12} and iron

2 *Defective absorption* is a common cause of nutritional anemia in adults In pernicious anemia vitamin B_{12} deficiency arises in this manner as a result of defective gastric function and the failure to secrete the "intrinsic factor" that is essential for the normal absorption of vitamin B_1 [84] A similar disorder is produced by total gastrectomy a procedure that removes the tissue that secretes the intrinsic factor In this circumstance the deficiency does not appear for several years after gastrectomy because the normal body reserves of vitamin B_1 do not become depleted for some time Macrocytic anemia associated with a megaloblastic type of erythropoiesis that is characteristic of vitamin B_{12} or folic acid deficiency sometimes occurs in sprue or other diseases accompanied by severe diarrhea or other abnormalities which may lead to malabsorption A similar anemia due to vitamin B_1 deficiency that is refractory to oral treatment yet responsive to parenteral therapy has been reported in patients with diverticula strictures and fistulas of the small intestine In such circumstances the bacterial flora of the gut seems to play an important role in some way possibly by inhibiting the absorption of vitamin B_1 or by competing for this vitamin since treatment with antibiotics may induce a remission accompanied by a return of the ability to absorb vitamin B_1 that is administered orally [41] Although total gastrectomy is rarely followed by an iron deficiency anemia this type of anemia often occurs several years after partial gastrectomy Investigation of such patients *has shown that the iron deficiency in this circumstance is probably at least* partly the result of an inability to absorb iron that is contained in food while the ability to absorb medicinal iron may be normal [73]

3 *Excessive demands* for erythropoiesis may produce a deficiency of essential factors if the reserve stores of the body have been depleted even though the normal dietary intake has been maintained During pregnancy *anemia sometimes occurs because of iron deficiency that is the result of the* added demands of fetal erythropoiesis a similar type of anemia is seen in some children because of the increased need for iron to meet the increase in blood volume that accompanies body growth A deficiency of folic acid or more rarely of vitamin B_{12} develops in some women during pregnancy

particularly in those who have a poor diet or suffer from severe gastrointestinal upsets Depletion of the body reserves of folic acid may be a factor in megaloblastic anemia of pregnancy but it has been pointed out that in man neither the daily requirement of folic acid nor the quantity of the body stores is known[37] The existence of some reserves of folic acid is shown by the observation that some expectant mothers do not develop megaloblastic anemia despite extremely poor diets

B *Bone Marrow Failure*

Even if adequate amounts of all the substances required for erythropoiesis are ingested and absorbed normally erythropoiesis may be inadequate because of defective function of the bone marrow Inadequate erythropoiesis is responsible for the occurrence of anemia in a number of circumstances such as (1) in the presence of other disease (2) after the ingestion of drugs (3) after exposure to chemicals (4) after irradiation (5) in endocrine diseases (6) in many patients the disease is idiopathic

1 *Diseases* of long duration such as chronic infections particularly if accompanied by fever and various metabolic disorders, such as the uremic state often depress erythropoiesis The decreased marrow activity is reflected by diminished iron utilization and reduced production of young red cells[36] Although all of the factors that are responsible for the depression of marrow activity are not known some important details have been established The plasma of uremic rabbits has been shown to contain a lower than normal quantity of the erythropoietic factors and uremic rabbits have been shown to be less responsive than normal to injection of plasma rich in erythropoietin[3] Bone marrow cultures from uremic patients have been shown to have lower iron utilization than cultures from normal individuals[81] In other diseases such as carcinomatosis, disseminated tuberculosis and leukemia, actual mechanical encroachment on the erythropoietic tissue may be a factor in the anemia although it is rarely the sole mechanism

2 *Drugs* act to depress the bone marrow in various ways Anemia may occur alone but more often it occurs in combination with leukopenia or thrombocytopenia Some drugs depress the bone marrow which is a tissue composed of rapidly dividing cells by their pharmacologic action which interferes with nucleic acid metabolism and inhibits cell division or maturation In this category are benzol the alkylating agents (nitrogen mustard chlorambucil triethylene melamine (T E M) Myleran) folic acid antagonists (aminopterin amethopterin) the purine antagonists (6 mercaptopurine) and urethane The effects of these drugs which are discussed in more detail in Chapter IX are predictable and quantitative although individual patients vary considerably with regard to the amount of drug that is required to produce the effects Other drugs produce depression of hematopoiesis only in certain susceptible individuals Marrow depression which may be temporary or permanent may occur without warning even after only a very small dose of the drug particularly if the individual has been

exposed to the drug previously Reactions of this type have been reported to follow exposure to a variety of coal tar products antibiotics anticonvulsants and heavy metals[69, 80]

3 *Chemicals* that are employed in various industries and occupations may damage the bone marrow in the same manner as chemicals used as drugs Usually an element of individual susceptibility is a factor The chemicals that have been incriminated most often are organic solvents particularly benzol and its derivatives[86] Exposure to chemicals often occurs through the use of cleaning agents or insecticide sprays

4 *Ionizing radiation* from x rays or radioactive materials causes anemia by depression of erythropoiesis if the bone marrow is exposed sufficiently During roentgen ray therapy or after the use of radioactive isotopes the full hematologic effect may not be evident until several weeks after exposure With the carefully calculated daily and total dosages that are used in irradiation therapy permanent damage rarely results because therapy is rarely continued for more than several weeks at a time However if large doses of irradiation are used as is sometimes the case when carcinoma is treated or if courses of therapy are repeated frequently over a period of years as happens in some patients with diseases of the lymphoma group the effects on the marrow may be so marked and persistent that they may limit or preclude further therapy of this type Although the exact mechanism of action of irradiation is incompletely understood at present it is known that cell multiplication is suppressed possibly by inhibition of nucleic acid synthesis[6] (See also Chapter IX)

5 *Hormones* Both the pituitary gland and the thyroid gland are important in erythropoiesis This is shown by the development of anemia after removal of either of the glands in animals and by the occurrence of anemia in patients with hypopituitarism and myxedema[9] It has been postulated that the anemia in myxedema which is rarely severe represents an adjustment to the decreased body metabolism comparable to the effect produced by exposure to increased oxygen tension rather than a deficiency of the thyroid hormone per se[9] The demonstration that patients with myxedema and anemia respond in a normal fashion to bleeding without thyroxin administration favors this interpretation[60] as does the observation that patients who have an associated deficiency of vitamin B_{12} or iron can respond to some extent to these substances without the administration of thyroxin[9] It has been shown that some individuals with myxedema absorb vitamin B_{12}[61] and iron poorly[3]

6 *Idiopathic" aplastic anemia* In some patients no explanation can be found for the bone marrow failure that is responsible for severe anemia There is no history of unusual exposure to drugs chemicals or irradiation and no evidence of an associated disease that might cause bone marrow depression A congenital defect has been postulated for the disorder that occurs in infants and very young children but aplastic anemia also develops in adults The marrow hypoplasia is usually associated with pancytopenia but in some patients anemia occurs alone in others the anemia is accompanied

by either leukopenia or thrombocytopenia Theoretically the marrow failure may be due either to the lack of some factor necessary for erythropoiesis or to the inability of the cells to respond to the factor or factors that normally stimulate erythropoiesis Against the first possibility is the evidence that the plasma of at least some adult patients with aplastic anemia contains large amounts of erythropoietin such plasma induces an erythropoietic response when injected into hypophysectomized test animals[39] These observations suggest that the hematopoietic cells have been damaged in some way and are unable to respond normally to stimuli The observation that extramedullary hematopoiesis rarely occurs in aplastic anemia and occurs often when *there is encroachment on the marrow by other tissue* such as carcinoma or fibrosis also favors this interpretation

II BLOOD LOSS

A *Acute blood loss* usually results in a normocytic, normochromic type of anemia Although the total blood volume falls immediately after hemorrhage the hematocrit determination may not reflect the degree of blood loss until 48 hours have elapsed In persons with a responsive bone marrow acute hemorrhage which affords a well known stimulus to erythropoiesis is usually followed by an increase in platelets leukocytes, and reticulocytes The rise in platelets and leukocytes which is greater with internal hemorrhage than with external hemorrhage occurs in a few hours The reticulocytes begin to increase in two or three days and reach a peak in five to ten days

B *Chronic blood loss* is a common cause of anemia Bleeding of this type nearly always results in a deficiency of iron which when severe is characterized by a hypochromic microcytic anemia associated with normoblastic erythropoiesis in the bone marrow In milder degrees of anemia due to deficiency of iron hypochromia and microcytosis may be absent[8] The reticulocyte count serum bilirubin level platelet count and leukocyte count are usually normal Any disorder which leads to chronic or repeated gastrointestinal or uterine bleeding may produce iron deficiency If the iron stores have been reduced previously by growth menstruation or pregnancy the actual blood loss need not be conspicuous in order to produce anemia Even the physiologic blood loss of menstruation may be sufficient to cause anemia *in young girls with borderline stores of iron Mild menorrhagia at the menopause* may produce the same result in older women

III INCREASED HEMOLYSIS

The normal survival time of the circulating erythrocyte as determined by the Ashby chromium[1] N[1] glycine and diisopropyl fluorophosphate methods is about 120 days The actual mechanisms by which the erythrocytes are destroyed even in normal circumstances are poorly understood The following mechanisms have been considered (1) erythrophagocytosis by cells of the reticuloendothelial system (2) osmotic lysis (3) destruction

by tissue lysins (4) mechanical fragmentation of old or damaged cells or (5) damage by agglutinins [8] In normal circumstances and in most instances of hemolytic disease destruction of the erythrocytes or at least the conversion of hemoglobin into bilirubin apparently takes place in the cells of the reticuloendothelial system In certain hemolytic anemias the erythrocytes are fragmented or lysed intravascularly and hemoglobin is released into the plasma Hemolytic anemia occurs whenever a decreased survival time of the erythrocytes is not balanced by increased erythropoiesis A decrease in survival time of the erythrocytes to less than 120 days may be necessary to produce anemia if the bone marrow is functioning normally since the bone marrow has the capacity to increase its production of erythrocytes six fold Unless erythropoiesis is depressed for some reason hemolytic anemia is characterized by both increased hemolysis and increased erythropoiesis Evidence of increased hemolysis is found in the increased excretion of fecal urobilinogen and urinary urobilinogen and in the elevation of indirect reacting bilirubin in the serum Increased erythropoiesis is manifested by hyperplasia of the bone marrow, reticulocytosis in the peripheral blood and increased iron utilization

Several different pathogenic mechanisms may cause a diminution in the survival time of erythrocytes These mechanisms can be conveniently classified as intracorpuscular" or "extracorpuscular" "Intracorpuscular defects which are intrinsic in the erythrocytes produced by the patient result in a diminished survival time of the patient s erythrocytes in his own circulation and also after transfusion into a normal compatible recipient "Extracorpuscular" factors are different in that they are part of the environment that the erythrocytes encounter in the patient s circulation normal erythrocytes have a diminished survival when transfused into the patient while the erythrocytes produced by the patient survive for the normal time when transfused into a normal recipient [9] Intracorpuscular defects are generally congenital the result of some inherited disorder Extracorpuscular defects are usually acquired as the result of some associated disease or disorder In some patients the hemolytic anemia is the result of a combination of intracorpuscular and extracorpuscular abnormalities

A *Intracorpuscular Defects*

1 *Hereditary spherocytosis* (congenital hemolytic jaundice) is inherited as a Mendelian dominant[9] and is characterized by erythrocytes that have a thickness/diameter ratio greater than normal The cells are abnormally susceptible to hypotonic saline[16 77] and the tendency of the cells to undergo lysis is increased by incubation [43 44] Perfusion experiments have demonstrated that the increased thickness of the erythrocytes leads to their sequestration in the spleen where they are destroyed [34 91 93] These studies have demonstrated that although there is an inherited "intrinsic" defect in the erythrocytes in hereditary spherocytosis, the decreased survival time of the red cells is the result of their sequestration and destruction by the spleen

and for this reason splenectomy cures the hemolytic anemia in nearly all patients even though the defect in the erythrocytes—spherocytosis—persists The actual spherocytosis may be a reflection of an inherited abnormality in phosphate partitioning associated with derangements in energy yielding glycolytic reactions [1 7] The finding of increased sodium transport across the surface membrane of the erythrocytes in hereditary spherocytosis—which did not correlate with the osmotic fragility autohemolysis or other tests—has led to the suggestion that hereditary spherocytosis is a composite of genetically determined biochemical abnormalities [6]

2 *Thalassemia* (Cooley s anemia Mediterranean anemia) is a rare inherited disorder that is found most often in families of Mediterranean origin It may be manifested by different syndromes that vary in severity from mild hematologic abnormalities to severe anemia Studies of families with this disorder led to the suggestion that the severe form of the disease represents homozygosity of the genetic factor (thalassemia major) while the milder state reflects heterozygosity of the genetic factor (thalassemia minor) [1 8] A prominent feature of the disorder is the occurrence of hypochromic microcytes that are abnormally thin (leptocytes) These cells have the appearance of target cells on the stained blood smear and are abnormally resistant to hypotonic saline [4 90] The alterations that are responsible for the leptocytosis and the method of destruction of the cells in the patient which occurs in the absence of the spleen are unknown Although the main defect in this disease is intracorpuscular in some patients there is an additional extracorpuscular mechanism associated with the splenomegaly that leads to premature destruction of even normal cells that are transfused [6] Hemoglobin analysis has revealed an increase in fetal hemoglobin (HbF) and hemoglobin A in the different thalassemic syndromes The percentage of hemoglobin F does not correlate with the clinical severity of the disorder [78] The hemoglobin F in thalassemia major has been found to range from 40 to almost 100 per cent In other milder cases the hemoglobin F usually ranges from 20 to 40 per cent [8] In some patients with only minimal evidence of thalassemia in the peripheral blood the level of hemoglobin F may be as high as 80 per cent [1] while in other similar cases it may not be detectable [78]

3 Hemoglobinopathies Pauling Itano Singer and Wells made the important discovery that the sickling phenomenon in sickle cell disease is due to a pathologic difference in the hemoglobin molecule that could be demonstrated by electrophoresis [70] The difference between these hemoglobin molecules is in the globin portion and not in the heme fraction This new concept that a disease can be an expression of a molecular disorder that is genetically controlled has opened up an entirely new field of investigation in hematology [6] ' Several excellent reviews of this important and rapidly changing field have been published [18 53 63 94]

The determination of the type of hemoglobin is genetically controlled and for a satisfactory explanation of the various combinations of normal and abnormal hemoglobins that have been reported it may be necessary to postulate at least three different genetic loci [67 68] When the abnormal hemo

A (M J)

C (patient)

S (L J)

A (C J)

A (F J)

Figure 18 Example of hemoglobin electrophoresis showing characteristic mobilities of homozygous hemoglobins A, C and S using filter paper as a supporting medium with a veronal buffer at pH 8 6 Cathode to the right.

globin occurs in the heterozygous state along with normal adult hemoglobin a non symptomatic "trait" condition usually results When the abnormal hemoglobin is present in the homozygous state or when an individual is heterozygous for two abnormal hemoglobin types a clinical disease characterized by a hemolytic disorder of the intracorpuscular type usually occurs (Fig 19) In addition to the normal adult hemoglobin[70] and fetal hemoglobin, F the following electrophoretically abnormal hemoglobins have been reported S[70] C[55] D E[19, 54] G[83] H[74] I[74] J[60] K[11] L N[5, 83] Q[63] "Norfolk"[1] "Barts"[1] Hemoglobins D G H I J K, L N Q "Norfolk" and "Barts" have been found only in the heterozygous or trait condition Disease states characterized by hemolytic anemia have been reported in the following hemoglobin combinations SS (sickle cell anemia) SC (sickle cell–hemoglobin C disease) S thalassemia (microdrepanocytic disease thalassemia-sickle cell disease) S D (sickle cell–hemoglobin D disease) CC (homozygous C disease) C Thalassemia (hemoglobin C–thalassemia disease) EE (homozygous E disease)

The chemical differences between hemoglobin S (and the other abnormal hemoglobins) and hemoglobin A are slight It is now known that of the

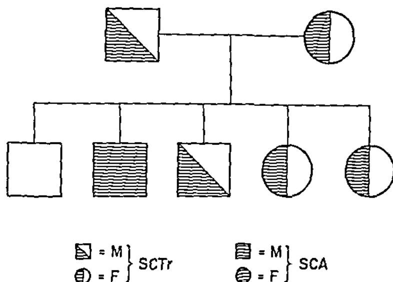


Figure 19 Heredity in sickle cell disease Study of the hemoglobin types in a family which illustrates the results that occurred from the mating of parents who were heterozygous for hemoglobin S

nearly 300 amino acids present in each of the identical half molecules of hemoglobin only one is abnormal in hemoglobin S In each half of the hemoglobin S molecule one normally occurring glutamic acid is replaced by valine with the net loss of two carboxyl groups by the entire molecule [51]

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demonstrated that the mechanical fragility of the erythrocytes in sickle cell anemia is increased when the cells are in the sickle form[31, 77]

4 Primaquine Sensitivity Primaquine sensitivity has been studied extensively and it has been shown that the hemolytic anemia induced by the drug is due to intracorpuscular defects that render the cells susceptible to the drug[3] This drug sensitivity which has a familial distribution is seen but rarely in Caucasians but occurs in about 10 per cent of the American Negroes[7] The primaquine induced hemolytic anemia is characterized by an acute hemolytic phase of about eight days duration which is followed first by a recovery phase and then by a phase of equilibrium when further administration of primaquine in large doses does not produce further hemolysis Survival studies in which young erythrocytes tagged with radioactive iron were utilized demonstrated that the hemolytic action of primaquine affected only older erythrocytes[7] The intrinsic defect in the erythrocyte is characterized by several biochemical abnormalities The following have been demonstrated (1) low reduced glutathione (2) unstable reduced glutathione that falls when a hemolytic drug is administered in vivo or when the erythrocytes are incubated with acetylphenylhydrazine in vitro (3) deficiency of glucose 6-phosphate dehydrogenase (4) increased glutathione reductase (5) increase in aldolase[3] A deficiency of glucose 6 phosphate dehydrogenase has also been found in 7 2 per cent of healthy Negroes and in 1 3 per cent of healthy Caucasians[35]

The studies on primaquine sensitivity have an importance beyond the problem of primaquine hemolytic anemia These investigations have established that an inherited abnormality in cellular physiology is responsible not only for primaquine sensitivity but also for the sensitivity that is concerned in the hemolytic anemia occurring in some individuals after the ingestion of sulfonamide drugs fava beans and other compounds such as phenylhydrazine and napthalene[79] The clinical disease occurs as a result of the action of one of the extrinsic factors on erythrocytes that are susceptible because of an intrinsic hereditary defect

5 Paroxysmal Nocturnal Hemoglobinuria (Marchiafava Micheli Syndrome) Paroxysmal nocturnal hemoglobinuria is a rare hemolytic disorder that is characterized by intravascular hemolysis and hemoglobinuria which is usually more marked during or immediately after sleep[4] It has been demonstrated that the increased hemolysis is due to an intracorpuscular defect since erythrocytes from patients with paroxysmal nocturnal hemoglobinuria have a diminished survival time when transfused into normal subjects while erythrocytes from normal individuals survive normally when transfused into patients with paroxysmal nocturnal hemoglobinuria[24, 7] Auto-survival studies on three patients utilizing Cr^{51} tagged cells have shown half survival times of 2 7 and 17 days however in each instance a portion of the cell population survived for prolonged periods and suggested the presence of a double population[50] Although the serum of patients with this disease is normal hemolysis of the abnormal erythrocytes occurs when the pH is reduced to a level of 6 7 to 7 1 This has suggested that the increased

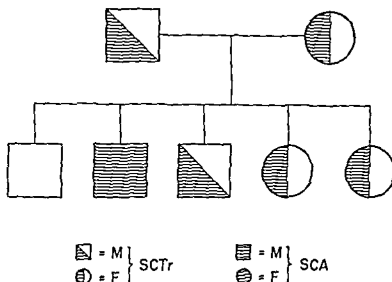


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countered in the circulation may lead to increased destruction It has also been suggested that the presence of the antibody on the cell membrane alters the metabolism at the cell surface and thereby causes irreversible changes in the membrane with the production of spherocytosis that leads to erythrocyte destruction in the spleen or in the circulation [4] The speed with which hemolysis was found to occur in some studies is evidence against the occurrence of this mechanism [5] Another possible explanation is that spherocytosis in vivo is caused by auto-agglutination and stagnation leading to ischemia of tissue cells which release substances that further damage the erythrocytes [14] Erythrophagocytosis by the leukocytes of the peripheral blood and by cells of the reticuloendothelial system may also be a mechanism of cell destruction It seems probable that the mechanism of destruction of the red cells is not the same in every case of "auto-immune" hemolytic disease It has been suggested that three pathways exist (1) intravascular destruction due to the presence of a complete "antibody" a hemolysin (2) lysis after sequestration primarily in the liver or lungs that occurs because of the presence of a complete "antibody" an agglutinin (3) lysis primarily in the spleen after sequestration that follows agglutination produced by an incomplete agglutinin plus other factors [67]

2 Iso immune Hemolytic Disease Iso-antibodies are the naturally occurring antibodies such as anti A and anti B and those that develop in response to the stimulus afforded by exposure to erythrocytes of other blood groups These antibodies *do not* affect the patient's own erythrocytes Iso immune hemolytic disease occurs as a result of the incompatibility of the blood group antigens It occurs as a result of mismatched transfusions involving the ABO system the Rh system and other groups when the individual's serum contains antibodies for the other blood group agglutinogens A similar mechanism is responsible for the hemolytic disease of the new born erythroblastosis fetalis This disease most commonly occurs when the mother is Rh negative (lacks the D antigen) while the fetus is Rh positive (has the D antigen) but it also occurs in some instances of ABO incompatibility The mother develops antibodies against the red cells of the fetus and the antibodies pass through the placenta to destroy erythrocytes in the fetal circulation The exact method by which the immunizing antigen enters the mother's circulation is not known but it is likely that the event occurs at parturition since the disease generally spares the first born child and affects later children Sensitization may also be present in nulliparous women because of previous blood transfusions However an incompatibility of the blood groups of mother and child does not necessarily mean that hemolytic disease will develop since antibodies are not always formed in sufficient quantity

3 **Paroxysmal Cold Hemoglobinuria** Paroxysmal cold hemoglobinuria is a very rare disease characterized by sudden attacks of intravascular hemolysis and hemoglobinuria It has been considered to be a manifestation of syphilis but its occurrence in patients in whom syphilis has been excluded has led to the suggestion that in some patients the positive serologic tests

hemolysis that occurs during sleep is due to carbon dioxide retention Studies have shown that for the in vitro destruction of erythrocytes in nocturnal hemoglobinuria the following are essential serum complement magnesium ion (Mg^{++}), pH between 6 7 and 7 1 properdin Properdin is a normal constituent of serum distinct from complement that is concerned in natural immune activities that do not require serum antibodies The lytic action of serum parallels the properdin content but the other factors are essential for the reaction [48, 49]

B *Extracorpuscular Defects*

1 Auto-immune Hemolytic Anemias Auto-immune hemolytic anemias are characterized by the production of "antibodies" that have the ability to act on the patient s own corpuscles In most instances these "auto-antibodies" also act as iso antibodies and react with other normal human erythrocytes to some degree Hemolytic anemias of this type are acquired and from a clinical standpoint may be classified as (1) idiopathic (cause unknown) or (2) symptomatic (occurring in association with some other disease such as malignant lymphoma leukemia virus pneumonia collagen disease) From a serologic standpoint the antibodies may be classified as "cold" antibodies which are markedly potentiated by cold or "warm" antibodies which act in vitro at 37 at least as well as at lower temperatures [1] The presence of the auto antibodies of both the warm and cold types (which are absorbed on the patient s erythrocytes) is shown best by the antiglobulin (Coombs) test During clinical remission the presence of auto-antibodies in the patient s serum can sometimes be demonstrated by testing with trypsinized cells when the indirect antiglobulin test is negative [23]

The factors that lead to the formation of the auto-antibodies in hemolytic anemia are not well understood Two mechanisms have been suggested (1) an alteration in the patient s erythrocytes that causes them to act as antigens and leads to a specific immune response by the patient and (2) the production of antibodies or substances that resemble antibodies as part of an outpouring of globulin from the antibody forming tissue of the patient in response to some other non specific stimulus The occurrence of this type of hemolytic anemia in patients who have disorders of the reticuloendothelial system the frequent association of increases in the serum globulin level and the frequent occurrence of other positive serologic reactions favor the production of abnormal protein as the fundamental disorder [3, 4] Furthermore classic types of immune red cell reactions have been observed when it was clear that an antibody had not developed against the unaltered red cell [57]

The mechanisms by which the erythrocytes are actually destroyed are not known with certainty The first phase in the destruction of the cells appears to be fixation of the globulin to the cell membrane [5] Since most auto antibodies are not lytic destruction probably occurs through other mechanisms The demonstration that agglutinated erythrocytes coated with antibody have an increased mechanical fragility suggests that the trauma en

countered in the circulation may lead to increased destruction It has also been suggested that the presence of the antibody on the cell membrane alters the metabolism at the cell surface and thereby causes irreversible changes in the membrane with the production of spherocytosis that leads to erythrocyte destruction in the spleen or in the circulation [4] The speed with which hemolysis was found to occur in some studies is evidence against the occurrence of this mechanism [5] Another possible explanation is that spherocytosis in vivo is caused by auto-agglutination and stagnation leading to ischemia of tissue cells which release substances that further damage the erythrocytes [11] Erythrophagocytosis by the leukocytes of the peripheral blood and by cells of the reticuloendothelial system may also be a mechanism of cell destruction It seems probable that the mechanism of destruction of the red cells is not the same in every case of "auto-immune hemolytic disease It has been suggested that three pathways exist (1) intravascular destruction due to the presence of a complete "antibody" a hemolysin (2) lysis after sequestration primarily in the liver or lungs that occurs because of the presence of a complete "antibody" an agglutinin (3) lysis primarily in the spleen after sequestration that follows agglutination produced by an incomplete agglutinin plus other factors [57]

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for syphilis are false positive reactions that have occurred in the presence of the antibody of paroxysmal cold hemoglobinuria.[3] The attacks are precipitated by chilling of the body and hemolysis occurs during the subsequent warming. Donath and Landsteiner demonstrated that hemolysis occurred in paroxysmal cold hemoglobinuria because of the presence of an autohemolysin in the patient's blood.[5] The hemolysin in the patient's plasma unites with the patient's erythrocytes in the presence of complement at temperature of 15° C or less. In the presence of complement hemolysis occurs on warming at a pH of 7.0 to 8.0. The hemolysin is not active at body temperature or at room temperature.[4] The paroxysmal cold hemoglobinuria antibody, which is the hemolysin, is a gamma globulin and erythrocytes that are sensitized by incubation in dilute paroxysmal cold hemoglobinuric serum are agglutinated by antiglobulin (Coombs) serum.[6] It has been suggested that the damaged erythrocytes are removed by erythrophagocytosis since this reaction has been demonstrated in vitro.[9]

4. **Infectious Agents.** Hemolytic anemia may occur as a result of a variety of infections. Among these are septicemia due to Cl. welchii, staphylococci, streptococci, and infections due to Vibrio cholerae. Hemolytic anemia has also been reported in various types of tuberculosis, bacterial endocarditis and influenzal meningitis. In malaria destruction of the erythrocytes occurs as a consequence of the localization of the parasites within the red cells. The severe, fulminating hemolytic anemia, "blackwater fever," that sometimes occurs in infections with Plasmodium falciparum may be due in part to antibodies that are produced as a result of quinine ingestion.[4] A severe acute hemolytic anemia occurs in Oroya fever, which is caused by infection with the Bartonella bacillus. The mechanism by which certain viruses cause hemolytic anemia appears to be through the production of autoantibodies rather than by a direct action of the virus on the erythrocytes.

5. **Chemical Agents.** A variety of chemical agents have been incriminated as causes of hemolytic anemia. Thirty-one different compounds have been listed by Wintrobe.[8] With some, such as phenylhydrazine and naphthalene (present in mothballs), the effect is related to the amount of the compound that is ingested. Other compounds, such as sulfonamides, quinine and primaquine, cause hemolytic anemia because of idiosyncrasy on the part of the patient. It now appears that in most individuals the susceptibility of the erythrocytes to these drugs and chemicals is the result of an inherited intracorpuscular defect.[79]

6. **Physical Agents.** Hemolytic anemia, which is sometimes severe, occurs in extensive burns. As a result of damage to the erythrocytes by heat the red corpuscles become more susceptible to the trauma in the circulation and to stagnation in the tissues.[4] Increased hemolysis has been demonstrated during the first week in burns and is severe in third degree burns of more than 20 per cent.[4] In addition, N^{15} glycine studies of hemoglobin production have shown that marked depression of erythropoiesis occurs about 10 days after the injury at a time when the hemolytic anemia might be expected to lead to increased erythropoiesis.[4, b]

7 Vegetable Poisons A severe hemolytic anemia known as favism occurs in some individuals as the result of the ingestion of the bean Vicia fava Most of the cases have occurred in Italy and Sardinia but cases have also been seen in other Mediterranean countries and the Middle East The hemolytic episodes often acute are sometimes fatal The direct and indirect antiglobulin (Coombs) tests are often positive at the time of an attack indicating the presence of incomplete warm antibodies on the erythrocytes and in the plasma[64] Favism was formerly considered to be a manifestation of allergic sensitization[3] Recent studies indicate that individuals who are susceptible to the fava bean have a constitutional anomaly of the erythrocytes glutathione deficiency similar to that found in patients who are sensitive to primaquine and other drugs[79] Since some persons apparently do not develop the hemolytic anemia until after the second exposure to the offending drug or the fava bean it has been postulated that at times both a glutathione deficiency and the sensitization to the noxious agent are necessary to produce hemolysis[79]

8 Miscellaneous Types of Hemolytic Anemia HYPERSPLENISM Hemolytic anemia sometimes develops in patients who have splenomegaly when no auto-antibodies or other abnormal hemolytic mechanisms can be demonstrated by the usual tests The usual features of hemolytic anemia may be present, but on occasions during the management of a patient with anemia by means of transfusions an increased demand for transfusions is the main clinical evidence of the hyperhemolysis In some patients leukopenia and thrombocytopenia of considerable severity also occur The reductions in the cellular elements of the circulating blood are associated with an apparently active bone marrow Splenectomy is sometimes beneficial and the syndrome has been termed hypersplenism" A primary disorder of this type appears to be extremely rare if it exists The syndrome is seen with splenomegaly that occurs in many different diseases such as cirrhosis portal vein thrombosis diseases of the lymphoma group collagen disorders fungus disease and tuberculosis The mechanism of the hyperhemolysis is unknown The increased destruction of the erythrocytes may be related to the circulatory stasis in the enlarged spleen in some way but the possibility of some humoral mechanism cannot be excluded

HEMOPATHIC HEMOLYTIC ANEMIA The term hemopathic hemolytic anemia has been suggested for the hemolytic anemia that occurs in various disease states such as uremia carcinoma leukemia and infections when no abnormal antibodies can be demonstrated[8] The anemia is the result of a shortened survival time of the erythrocytes associated with an inadequate marrow response The presence of a hemolytic element has been established by cell survival studies and the subnormal response of the marrow has been shown by the absence of reticulocytosis or increased iron utilization The survival curve of the erythrocytes in this type of anemia is linear and resembles a normal curve except for its slope For this reason it has been postulated that the increased cell destruction reflects premature senescence of the erythrocytes as the result of abnormal metabolism which occurs in the pri

mary disease. It has been suggested that such metabolic abnormalities may lead to an early dissipation of the enzymes that are responsible for maintaining the normal integrity of the red blood cells.[8] Increased hemolysis also occurs in some patients with "aplastic" or "refractory" anemia but in such patients the hemolytic element is a minor factor compared to the failure of the bone marrow.[10, 60]

HEMOLYTIC ANEMIA ASSOCIATED WITH LIVER DISEASE. An acute type of hemolytic anemia has been observed in acute hepatic necrosis without evidence of auto immunization.[63] Chronic hemolytic anemia that is due to an extracorpuscular mechanism has been found to be the commonest type of anemia in chronic liver disease.[15, 70] The spleen is apparently the main site of hemolysis in these patients.

DIAGNOSIS OF THE PATIENT WITH ANEMIA

The objectives in the management of the patient with anemia are first the discovery of the cause of the anemia and second correction of the underlying defect. It is important to remember that the demonstration of "anemia" does not constitute a diagnosis but merely the recognition of a sign comparable to fever or edema. For this reason the seriousness of the anemia in any patient depends on the underlying cause of the anemia rather than the severity of the anemia. A mild degree of anemia caused by a carcinoma of the gastrointestinal tract is a serious affair for the patient while a severe degree of anemia from hemorrhoidal bleeding is not. Treatment of a patient with iron or vitamins or blood transfusions *without first making a diagnosis* other than "anemia" may jeopardize his chances of recovery. Anemia that is the result of iron deficiency secondary to blood loss from a carcinoma of the cecum may respond to iron salts orally or to blood transfusions while the carcinoma changes from a curable to an incurable lesion. In situations where the underlying cause cannot be corrected, accurate diagnosis is still essential for proper management. Unless this fact is recognized a *patient with a macrocytic anemia may be "cured" of his anemia* by treatment with the folic acid present in many multivitamin preparations but the central nervous system lesions may progress and become irreversible if the patient is suffering from the vitamin B_{12} deficiency of pernicious anemia.

A systematic investigation of the patient with suspected anemia nearly always provides the information necessary for the correct diagnosis and

proper treatment This investigation aims to provide answers to the following questions

1 Does the patient have anemia?
2 What are the morphologic characteristics of the anemia?
3 What is the mechanism of the anemia?
4 What is the underlying cause or disease that is responsible for the abnormal mechanism?
5 What is the best form of treatment?

Although it is at times impossible to establish a complete diagnosis in a patient with anemia in the vast majority of patients it is possible to classify the anemia according to the responsible mechanism as due to either deficient production blood loss or increased hemolysis This is usually possible by the use of relatively simple laboratory procedures that can be performed in the physician s office or the hospital laboratory If the physician understands the mechanism that is responsible for the anemia he is in a position to manage the patient intelligently by administering the proper treatment or by planning any further investigation that may be necessary to identify the disorder that is responsible for the abnormal mechanism

The answer to the question Does the patient have anemia? often cannot be gained from the history or the physical examination Anemia of moderate severity may not be detected even by careful inspection of the skin and mucous membranes In another circumstance a neurasthenic individual with pallor of the skin and mucous membranes may report all of the symptoms of anemia even though the blood is entirely normal The presence of anemia can be established only by laboratory procedures The simplest and most reliable screening procedure is the hematocrit determination[8] The normal range for adult males is 40 to 54 (47.0 ± 7.0) per cent and for adult females 37 to 47 (42.0 ± 5.0) per cent[7] The hemoglobin determination which has an accuracy comparable to that of the hematocrit if it is performed properly is also advocated as a screening procedure by some authors[8b] In our experience the hematocrit determination is more accurate than the determinations of the levels of hemoglobin or erythrocytes as these are usually performed but even this procedure is far from infallible Misleading values may be obtained if the blood is drawn from a vein subjected to too much stasis if an improper amount of anticoagulant is used or if centrifugation is faulty The volume of packed cells is also influenced by the osmotic pressure in the plasma low levels of serum electrolytes cause a rise in the hematocrit while elevations cause a fall (Fig 20) Even if the techniques are not faulty values in the normal range may be obtained occasionally in patients with either macrocytic anemia or iron deficiency anemia of mild degree We have seen a patient with pernicious anemia and involvement of the central nervous system who had a hematocrit of 42 per cent a hemoglobin of 14.3 gm per 100 ml and an erythrocyte count of 3.3 mil per cu mm An analogous difficulty has been encountered in patients with iron deficiency when the hematocrit was 40 per cent red blood count 5.0 mil per cu.mm and the

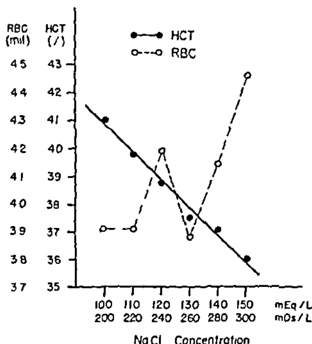


Figure 20 Effect of changing osmotic pressure on the hematocrit A single sample of blood was centrifuged and the plasma was removed and discarded The red cells were divided into six aliquots and the samples were restored to the original volume with varying saline concentrations as noted

hemoglobin 8 0 gm per 100 ml Such unusual occurrences do not detract seriously from the usefulness of the hematocrit as a screening test because the use of either the hemoglobin determination or erythrocyte count alone as a screening procedure is subject to the same limitations

In order to answer the question "What is the mechanism responsible for the anemia?" a careful history physical examination and certain laboratory tests that reveal the morphologic and other characteristics of the blood are necessary Laboratory procedures have assumed the most prominent place in the diagnosis of hematologic problems but this does not mean that a carefully taken history is not important Without the benefit of a complete history the laboratory results may be puzzling or even misleading

The History When the history is obtained the patient should be allowed to give his account of his illness first and then he should be questioned about various details A family history of anemia or splenectomy or a past history of recurrent episodes of anemia may be helpful particularly if the patient has hemolytic anemia The dietary history is important in children in pregnancy and in chronic illness for it is in these circumstances that a dietary deficiency is most likely to become manifest The history of the use of "tonics" and vitamins may help clarify an anemia that is difficult to classify

because the hematologic features characteristic of the underlying disease or syndrome have been altered by such preparations Repeated exposures to drugs or chemicals encountered in the patient's occupation or in the pursuit of a hobby may be a factor in the causation of anemia A careful review of the body systems should be part of the history taking because systematic questioning of this type may elicit important symptoms such as paresthesias mild ataxia excessive menstrual bleeding or even tarry stools that have not been volunteered by the patient

Physical Examination Although the physical examination alone rarely if ever suffices for the complete diagnosis of anemia nearly any part of the examination may contribute important information The appearance of hemorrhages and exudates in the fundi may be the first indication that the patient has bacterial endocarditis leukemia uremia or aplastic anemia The presence of tumor masses lymphadenopathy hepatomegaly or splenomegaly may bring the diseases of the reticuloendothelial system into consideration for the first time *Most hemolytic anemias with the exception of* sickle cell anemia are associated with splenomegaly Splenomegaly is unusual in untreated aplastic anemia and the detection of an enlarged spleen makes some other diagnosis more likely An appraisal of the nutritional state of the patient the color of the tongue the appearance of the papillae the presence of fissuring of the lips and the texture of the fingernails may give clues to the presence of a deficiency of folic acid, vitamin B_{1} or iron The detection of telangiectases on the skin or mucous membranes may give the first clue to an explanation of gastrointestinal hemorrhage Even the general appearance of the patient the tone of his voice and his manner of speaking may lead to the recognition of myxedema as the cause of an undiagnosed case of anemia that has proved refractory to the usual forms of antianemic therapy The demonstration of mild ataxia or decreased vibratory sense in the extremities may be the first indication that the patient has pernicious anemia particularly if the hematologic picture has been altered by inadequate specific therapy or by coexisting iron deficiency

Laboratory Studies Examination of the peripheral blood by laboratory methods is a most important step in the identification of the mechanism that is responsible for the anemia If the hematocrit value is low the following procedures are indicated determination of the hemoglobin erythrocyte count leukocyte count and a study of the stained blood smear The study of a film of blood that is properly made and stained gives valuable information about the size shape and hemoglobin content of the erythrocytes the level of the blood platelets and the type of leukocyte reaction A stain for reticulocytes affords a generally reliable indication of the erythropoietic activity of the bone marrow With the results of the hemoglobin hematocrit and erythrocyte determination the mean corpuscular volume (MCV), *mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin con*

centration (MCHC) can be determined and the anemia can be classified according to cell size and hemoglobin content as

macrocytic (MCV > 94 cμ) (MCHC $> 30\%$)
normocytic (MCV 80–94 cμ) (MCHC $> 30\%$)
microcytic (MCV < 80 cμ) (MCHC $> 30\%$)
hypochromic (MCV > 80 cμ) (MCHC $< 30\%$)
hypochromic microcytic (MCHC $< 30\%$) (MCV < 80 cμ)[87]

Table 6 *Laboratory Procedures Often Helpful in the Study of a Patient with Anemia*

USUAL INITIAL STUDIES
- Hematocrit determination
- Hemoglobin determination
- Erythrocyte count
- Reticulocyte count
- Study of stained blood smear
- Leukocyte count and differential count
- Platelet count (or estimate from blood smear)

SUSPECTED IRON DEFICIENCY
- Stool for occult blood
- Serum iron and latent iron binding capacity
- Stain of marrow prepartions for iron
- Roentgenographic study of the gastrointestinal tract

SUSPECTED VITAMIN B_{12} OR FOLIC ACID DEFICIENCY
- Bone marrow
- Gastric analysis after histamine injection
- Vitamin B_{12} absorption test (radioactive cobalt)
- Serum vitamin B_{12} level
- Roentgenographic study of the gastrointestinal tract

SUSPECTED HEMOLYTIC ANEMIA
- Bone marrow
- Serum bilirubin level
- Fecal urobilinogen excretion
- Test for sickling
- Hemoglobin electrophoresis
- Antiglobulin (Coombs) test
- Hypotonic saline fragility test
- Alkali denaturation test
- Hemoglobin determination, plasma and urine

SUSPECTED APLASTIC ANEMIA
- Bone marrow (aspiration or biopsy)
- Skeletal roentgenograms
- Serum iron and latent iron binding capacity
- Mediastinal roentgenograms for thymoma

OTHER TESTS OFTEN USED ESPECIALLY TO DIAGNOSE THE PRIMARY DISEASE
- L.E. preparation
- Blood urea
- Tissue biopsy (skin, lymph node, liver)
- Peroxidase stain
- Plasma electrophoresis
- Urine electrophoresis
- Bone marrow

The method of calculating these values is given on page 70 The characterization of the anemia as macrocytic normocytic microcytic or hypochromic microcytic provides a guide to other appropriate studies that are necessary to determine the mechanism and cause of the anemia The determination of the cell size and hemoglobin content by such calculations does not mean that a careful study of the blood smear is unimportant study of the smear gives valuable information that may not be apparent in the calculations A list of the studies that are often helpful is presented in Table 6

MACROCYTIC ANEMIA

If the patient is found to have macrocytic anemia of significant degree (MCV > 100 c μ) it is unlikely that acute hemorrhage has occurred and for practical purposes chronic blood loss may be eliminated from consideration A macrocytic anemia is usually due primarily to deficient blood production Less often a hemolytic mechanism is responsible Macrocytic anemia is most likely to be seen in the following circumstances (1) deficiency of vitamin B_{12} or folic acid (2) "aplastic" anemia (3) myxedema, and (4) hemolytic anemia

Deficiency of Vitamin B_{12} and Folic Acid

If the macrocytosis is due to a deficiency of vitamin B_{12} there is usually leukopenia and thrombocytopenia often of mild degree If the anemia is moderate or severe in degree study of the blood smear reveals considerable anisocytosis and poikilocytosis and the presence of large multisegmented neutrophils In the untreated patient the normal or subnormal reticulocyte count reflects the failure of a normal response to anemia The slightly elevated level of indirect reacting serum bilirubin and the increased fecal urobilinogen excretion reflect the increased destruction of erythrocytes in the peripheral circulation or in the bone marrow The occurrence of the megaloblastic type of erythropoiesis in the bone marrow establishes the presence of a deficiency of vitamin B_{12} or folic acid almost unequivocally

Since deficiencies of vitamin B_{12} and of folic acid can give identical hematologic pictures further study is necessary to determine which is responsible In this connection the gastric analysis is a very helpful procedure With but rare exceptions patients with pernicious anemia do not secrete hydrochloric acid after the administration of histamine and the presence of free HCl in the gastric analysis can almost be considered to exclude pernicious anemia from consideration The other causes of vitamin B_{12} deficiency such as total gastrectomy intestinal shunts fistulae and malabsorption syndromes are usually suggested by the history and confirmed by appropriate examinations If the patient has evidence of posterolateral sclerosis associated with a normocytic or macrocytic anemia and histamine fast achlorhydria the diagnosis of pernicious anemia should be strongly suspected even though the marrow is not megaloblastic

Folic acid administration can modify the appearance of the bone marrow and the peripheral blood in a patient with pernicious anemia while allowing the central nervous system lesions to progress. For this reason measurement of the level of vitamin B_{12} in the serum or use of one of the tests of vitamin B_{12} absorption such as the Schilling test that utilize vitamin B_{12} labeled with radioactive cobalt may be necessary to establish the diagnosis. In the Schilling test[70a] a measured amount of radioactive B_{12} is administered by mouth and a large "flushing" dose (1000 micrograms) of non-radioactive vitamin B_{12} is administered parenterally. Urine is collected and the amount of vitamin B_{12} absorbed from the gastrointestinal tract is measured by determining the amount of radioactive vitamin B_{12} that is excreted in the urine. If subnormal amounts of B_{12} are absorbed and excreted the test is repeated and intrinsic factor is administered with the oral vitamin B_{12}. The addition of the intrinsic factor leads to substantial improvement in absorption of vitamin B_{12} in patients with pernicious anemia but has little or no effect in those with malabsorption syndrome, shunts, and fistulae. If the patient has anemia and a megaloblastic bone marrow that are due to a deficiency of vitamin B_{12} the fact can be established if the administration of vitamin B_{12} parenterally produces a conversion of the megaloblastic erythropoiesis to the normoblastic type followed by a reticulocytosis and a rise of the hemoglobin and erythrocyte levels to normal.

When folic acid deficiency is responsible for the macrocytic anemia there *is usually a history of inadequate diet or some disorder such as a malabsorption* syndrome that produces a secondary deficiency. A dietary deficiency sufficient to produce this type of anemia is rare in the United States except in infancy, during pregnancy, in food faddists and in alcoholics. Folic acid deficiency may produce the same hematologic picture as vitamin B_{12} deficiency but the neurological lesions that are so common in vitamin B_{12} deficiency are usually absent or mild in folic acid deficiency. The occurrence of a macrocytic anemia and a megaloblastic marrow in an adult who has retained the ability to secrete free HCl strongly suggests the possibility of folic acid deficiency.

Aplastic Anemia

Mild or moderate macrocytosis is slightly more common than normocytic anemia in aplastic anemia. Although anemia of this type is usually accompanied by definite leukopenia and thrombocytopenia, anemia may occur alone. The term "chronic erythrocytic hypoplasia" is sometimes used to distinguish patients of the latter type. The reticulocyte count and serum bilirubin level are usually normal or subnormal in chronic erythrocytic hypoplasia; *the reticulocyte count is often 0 or 0.1 per cent.* A slight increase in the percentage of reticulocytes is occasionally seen but when this occurs the absolute value (the number of reticulocytes per cu mm) is usually normal. The macrocytic anemia of aplastic anemia even if severe is rarely accompanied by a significant degree of poikilocytosis or anisocytosis. The

bone marrow examination nearly always reveals the normoblastic type of erythropoiesis but in rare cases a megaloblastic marrow occurs Usually the marrow of patients with aplastic anemia is hypocellular often markedly so but the presence of normal or increased cellularity does not exclude the diagnosis Recognition of the cause of anemia of this type usually comes from the appropriate laboratory examinations At times the history of exposure to irradiation chemicals or drugs is helpful On occasion examination of the bone marrow in a patient with suspected aplastic anemia establishes a definite diagnosis of some other primary disease such as multiple myeloma leukemia carcinomatosis or tuberculosis In the majority of patients with aplastic anemia the cause remains obscure and the anemia is classified as "idiopathic"

Myxedema

A mild or moderately severe macrocytic anemia occurs commonly in patients with myxedema The red blood count usually is over 3 0 mil per cu mm The peripheral blood shows no appreciable poikilocytosis or anisocytosis and is generally normal in other respects The erythropoiesis in the bone marrow is normoblastic and the cellularity is either normal or slightly reduced The diagnosis of myxedema is usually suggested by the clinical features of the disease and is confirmed by the appropriate laboratory tests such as determinations of the serum cholesterol level basal metabolic rate radioactive iodine uptake and measurement of the plasma bound iodine If vitamin B_{12} deficiency occurs in association with the myxedema the anemia is more severe and the clinical and hematologic features of pernicious anemia are found

Hemolytic Anemia

A macrocytic type of anemia is not uncommon in hemolytic disorders particularly in the extracorpuscular or acquired type The diagnosis of the patient with hemolytic anemia is considered in the discussion of hemolytic normocytic anemias

NORMOCYTIC ANEMIA

If the patient is found to have a normocytic type of anemia (MCV 80 94 c μ) hyperhemolysis acute blood loss and deficient blood production must be considered

Hyperhemolysis

A reticulocyte count greater than 4 per cent in a patient who has neither had a recent hemorrhage nor received antianemic therapy should raise the suspicion of a hemolytic anemia Often the reticulocyte count is over 10 per cent, and sometimes it is 30 per cent or more Study of the stained smear

often reveals suggestive evidence of a hemolytic process in the form of nucleated red cells polychromatophilia punctate basophilia target cells or spherocytes Leukocytosis is often present These abnormalities in the peripheral blood reflect the hyperplasia that is evident on examination of the bone marrow At times the increase in erythropoiesis is so marked that a differential cell count of the marrow reveals that the myeloid erythroid (M E ratio) is 1 1 or less (normal 3 1 to 10 1) *In some patients with hemolytic anemia striking hyperplasia occurs* and the early forms of normoblasts become so numerous that the normoblastic nature of the erythropoiesis may be misinterpreted as megaloblastic because of the morphological similarities between the youngest cells of both series However careful study of the cells at all different stages of development particularly at the polychromatophilic stage nearly always enables one to interpret the marrow properly

Evidence of the hyperhemolysis is found in the moderate elevation of the indirect reacting bilirubin (1 0 to 4 0 mg per 100 ml) and in the increased fecal urobilinogen excretion Measurement of the fecal urobilinogen may be a very useful test in suspected hemolytic disease even though the wide normal range of daily variations from 40 to 280 mg seriously limits the usefulness of the test as an accurate measure of hemoglobin destruction When the mechanism of the anemia is not apparent particularly in patients with mild degrees of anemia the demonstration of an increase in the average 24 hour fecal urobilinogen excretion over a three or four day period may *establish the presence of increased hemoglobin breakdown In interpreting* the values obtained for the 24 hour fecal urobilinogen excretion it is important to remember that the fecal urobilinogen excretion may be reduced by antibiotics that change the bacterial flora of the intestinal tract and may be increased by recent blood transfusions The amount of urobilinogen excreted in the feces in 24 hours also depends on the total amount of circulating hemoglobin because a fixed percentage of the circulating hemoglobin is destroyed each day as a rule The mass of the circulating hemoglobin can be approximated by determining the number of grams of hemoglobin per 100 ml of blood and the body weight The following formula suggested by Dameshek[8] is helpful in interpreting the significance of the average daily fecal urobilinogen excretion in patients with anemia

$$\frac{\text{Normal Hb (15 gm 100 ml)}}{\text{Pt's Hb (gm/100 ml)}} \times \frac{\text{Normal weight (70 kg)}}{\text{Pt's weight}} \times \text{daily f u output (mg)} = \text{corrected daily fecal urobilinogen output (mg)}$$

The hemolytic index was also devised by Miller Singer and Dameshek[64] to relate the fecal urobilinogen excretion to the circulating mass of hemoglobin It is calculated as follows

$$\frac{\text{avg (of 4 days) daily output of fecal urobilinogen (mg)} \times 100}{\text{total Hb (Hb gm 100 ml)} \times \text{total blood volume/100}}$$

The hemolytic index *indicates the amount of urobilinogen derived daily* from 100 grams of hemoglobin The normal index is from 11 to 21

Although recognition of the hemolytic process is easy as a rule it is sometimes difficult because all the signs of increased hemolysis and increased erythropoiesis may be absent or inconspicuous if the patient is seen during an aplastic crisis which occasionally develops during an intercurrent infection

If the anemia is established as hemolytic in type further tests are necessary to determine the nature of the hemolytic disorder These include the antiglobulin (Coombs) test hypotonic saline fragility test tests for sickling and hemoglobin electrophoresis occasionally erythrocyte survival determinations and other studies may be employed Most of the important hemolytic anemias due to intracorpuscular defects are inherited and can be identified by the demonstration of characteristic abnormalities in the erythrocytes At times the study of parents or siblings is helpful in establishing the diagnosis Most instances of hemolytic anemia of the extracorpuscular variety are acquired and can be recognized by the demonstration of an auto immune mechanism or the coexistence of some other disease

Sickle cell disease is a disease of the Negro race almost exclusively Splenomegaly is rarely seen in adults with this disease The presence of the abnormal hemoglobin HbS can be demonstrated by placing the erythrocytes in moist preparations under a cover slip on a glass slide and sealing the cover slip by rimming the edges with petrolatum or by exposing the erythrocytes to freshly prepared reducing agents such as 2 per cent solution of sodium bisulfite ($NaHSO_3$) Sickle cell trait the heterozygous condition can be distinguished from sickle cell anemia the homozygous state by the lack of evidence of hyperhemolysis in patients with the trait and by means of hemoglobin electrophoresis By this technique the hemoglobin of cells from individuals with sickle cell trait can be shown to contain both normal hemoglobin (HbA) and sickle cell hemoglobin (HbS) while the erythrocytes of patients with sickle cell anemia appear to contain only sickle cell hemoglobin (SS) By means of hemoglobin electrophoresis patients with sickle cell–hemoglobin C disease (SC) homozygous hemoglobin C disease (CC) and other disorders can be identified Sickle cell–hemoglobin D disease can not be distinguished from sickle cell disease by electrophoresis as usually performed because hemoglobin S and hemoglobin D migrate similarly The presence of hemoglobin C thalassemia or hemoglobin D is often suggested by the presence of a large number of target cells in the stained smear of peripheral blood The severity of the anemia that is found in a patient who has a positive sickling test suggests various diagnostic possibilities Individuals with sickle cell trait have no anemia unless there is some associated disorder patients with sickle cell anemia usually have a severe degree of anemia (Hb 6 5 to 7 5 gm per 100 ml) a moderate anemia (Hb 8 0 to 10 0 gm per 100 ml) is usual in persons with sickle cell anemia–hemoglobin C disease and other double heterozygous conditions

Hereditary spherocytosis is rare in Negroes Splenomegaly is nearly always present The erythrocytes in this disorder the "spherocytes" are thicker than normal and have a decreased diameter The diagnosis frequently can

often reveals suggestive evidence of a hemolytic process in the form of nucleated red cells, polychromatophilia, punctate basophilia, target cells or spherocytes. Leukocytosis is often present. These abnormalities in the peripheral blood reflect the hyperplasia that is evident on examination of the bone marrow. At times the increase in erythropoiesis is so marked that a differential cell count of the marrow reveals that the myeloid-erythroid (M:E ratio) is 1:1 or less (normal 3:1 to 10:1). In some patients with hemolytic anemia striking hyperplasia occurs and the early forms of normoblasts become so numerous that the normoblastic nature of the erythropoiesis may be misinterpreted as megaloblastic because of the morphological similarities between the youngest cells of both series. However, careful study of the cells at all different stages of development, particularly at the polychromatophilic stage, nearly always enables one to interpret the marrow properly.

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$$\frac{\text{Normal Hb (15 gm. 100 ml.)}}{\text{Pt's Hb (gm./100 ml.)}} \times \frac{\text{Normal weight (70 kg.)}}{\text{Pt's weight}} \times \text{daily f.u. output (mg.)} = \text{corrected daily fecal urobilinogen output (mg.)}$$

The hemolytic index was also devised by Miller, Singer, and Dameshek[64] to relate the fecal urobilinogen excretion to the circulating mass of hemoglobin. It is calculated as follows:

$$\frac{\text{avg. (of 4 days) daily output of fecal urobilinogen (mg.)} \times 100}{\text{total Hb (Hb gm./100 ml.)} \times \text{total blood volume}/100}$$

The hemolytic index indicates the amount of urobilinogen derived daily from 100 grams of hemoglobin. The normal index is from 11 to 21.

Acute Blood Loss

The anemia that follows acute blood loss is nearly always normocytic If the acute hemorrhage is external and results from trauma or disease its occurrence is generally recounted in the history If the hemorrhage occurs from the gastrointestinal tract the event may not be noticed by the patient and the finding of anemia may be the first clue to its occurrence In some patients hemorrhages from a duodenal ulcer or a lesion of the small intestine recur over a period of years and cause anemia that can be puzzling if the patient does not consult his physician until after the occult blood has disappeared from the feces An elevated reticulocyte count in the absence of an increase in the serum bilirubin suggests recent hemorrhage if the patient has not recently been given specific therapeutic agents such as iron or vitamin B_1 Leukocytosis and thrombocytosis in the peripheral blood and marked normoblastic hyperplasia in the bone marrow may also afford clues to a recent hemorrhage The diagnosis of gastrointestinal bleeding is usually made from the history and by the detection of blood in the feces The demonstration that a gastrointestinal lesion is the source of the bleeding is not always easy and may not be possible without repeated roentgenographic study of the gastrointestinal tract or periodic observation of the patient In the unusual circumstances where the hemorrhage occurs into the muscles or other tissues as a result of trauma or ascorbic acid deficiency reticulocytosis may occur in association with an elevation of the serum bilirubin and suggest the presence of hemolytic anemia

Deficient Blood Production

Anemia that is the result of deficient blood production is nearly always normocytic or macrocytic but occasionally is slightly microcytic If the anemia is due to diminished erythropoiesis alone or to mild hyperhemolysis associated with depressed erythropoiesis as sometimes occurs in patients with malignancies and other diseases only the erythrocytes are reduced The leukocytes and platelets are usually normal unless altered by the primary disease It is unusual for anemia of this type to be the primary problem in diagnosis because the underlying disease such as chronic infection arthritis renal disease malignancy or endocrine disturbance is usually apparent More important is the fact that anemia of this type is sometimes the first clinical indication of the underlying disease Some ambulatory patients with renal disease present as problems of anemia because the slowly progressing renal insufficiency has not been recognized or suspected uremia is probably not the explanation of anemia unless the renal disease is chronic and severe The diagnosis of aplastic anemia which is often normocytic is discussed with the macrocytic anemias

A normocytic anemia also occurs in some patients with mild anemia associated with iron deficiency This may occur as an intermediate stage in the development of severe iron deficiency that is characterized by hypo

be suspected after an examination of the blood smear since the cells often appear smaller than normal, show little or no poikilocytosis and have little or no central pallor. However, the diagnosis of hereditary spherocytosis cannot be made with certainty from the blood smear alone because spherocytes may also be seen in other types of hemolytic anemia. The osmotic fragility test is employed to demonstrate the increased osmotic fragility that is characteristic of spherocytes. In this test samples of blood from the patient and from a normal control subject are placed in tubes containing aqueous solutions of sodium chloride of different concentrations. The concentrations of saline in the tubes where hemolysis begins and where it is complete are noted. Because of their shape spherocytes begin to hemolyze at higher concentrations than normal biconcave erythrocytes do. In the vast majority of patients with hereditary spherocytosis hemolysis begins and is complete at higher dilutions than is the case in normal blood. In normal blood hemolysis usually begins at 0.44 per cent and is complete at 0.32 per cent; in hereditary spherocytosis hemolysis often begins in concentrations of 0.50 to 0.70 per cent and is complete at 0.40 per cent. A twenty-four hour period of incubation may be necessary to demonstrate the increased osmotic fragility in some patients with hereditary spherocytosis.[8] The demonstration of an abnormal osmotic fragility test in other members of the family may be helpful in establishing the diagnosis in some patients with equivocal findings. The presence of increased susceptibility to hypotonic saline is not diagnostic of hereditary *spherocytosis; the test may be positive in other hemolytic disorders if spherocytes* are present in appreciable numbers.

Hemolytic anemia due to an extracorpuscular defect may be macrocytic or normocytic. In some patients the disease appears to be due to an auto-immune mechanism and in others to some other mechanism. If the anti-globulin (Coombs) test is positive auto-immune hemolytic disease is likely even though the osmotic fragility test reveals abnormal susceptibility to hypotonic saline. In such circumstances further study is necessary to determine if the hemolytic process is "idiopathic" or "secondary" to some underlying disease such as one of the lymphoma group, carcinoma, sarcoidosis or one of the collagen disorders. A careful diagnostic study that may include roentgenographic examinations, serum protein studies, a search for L.E. cells, and biopsy of a lymph node or other tissue is often necessary. A diagnosis of the idiopathic type of disease is reached by exclusion. At times the diagnosis of auto-immune hemolytic disease as either idiopathic or symptomatic can be established only after a prolonged period of observation and repeated examinations. The macrocytic anemia of chronic liver disease is usually due to an extracorpuscular type of hyperhemolysis but the Coombs test is usually negative. When the hemolytic anemia is due to chronic liver disease abnormal liver function tests and clinical evidence of liver disease are usually present. In other disease states a hemolytic element may be present but demonstrable only by careful study; in these patients the hemolytic process is usually mild and inadequate blood production is the most important mechanism.

blood loss Blood loss usually occurs because of some ulcerated lesion in the gastrointestinal tract Esophageal lesions hiatus hernia gastric carcinoma benign gastric ulcer duodenal ulcer tumor of the small intestine carcinoma of the colon and hemorrhoids are all possible sources of chronic blood loss Should the examination reveal no satisfactory source of bleeding the patient should be reexamined periodically and the roentgenographic studies repeated because any of the lesions particularly carcinoma of the cecum and stomach and tumors of the small intestine may be a source of bleeding even though they are not recognized on the initial roentgenographic study A diaphragmatic hernia can be responsible for chronic blood loss but this lesion is found so frequently in patients in the middle aged and older groups that it should not be accepted as the explanation of the anemia unless careful study of the remainder of the gastrointestinal tract has disclosed no other lesion Although iron deficiency can occur as the result of repeated bleeding from the genitourinary or respiratory tract bleeding from these sites of sufficient degree to cause anemia is unusual When bleeding of this type occurs it is readily apparent to the patient

In women menstruation and pregnancy afford added opportunities for the development of iron deficiency since the increased need for iron in these circumstances may not be met by increased absorption of dietary iron Even mildly excessive menstrual bleeding may lead to iron deficiency if the iron stores are low If the individual has low iron stores early in life the iron reserves may not be restored to normal by the diet during growth and adolescence In some premenopausal women it may be difficult to decide whether the number of pregnancies and the amount of menstrual bleeding can be accepted as a satisfactory explanation of the iron deficiency If there is any reasonable doubt repeated tests for occult blood in the feces and study of the gastrointestinal tract for a possible source of bleeding are indicated

TREATMENT IN RELATION TO DIAGNOSIS

The purpose of the diagnostic study is to enable the physician to decide what is the best form of treatment for the patient The most desirable form of treatment is the removal or correction of the cause When this cannot be accomplished therapy is directed toward the disordered mechanism For either form of therapy it is important to establish an accurate diagnosis before treating the anemia unless the patient's condition demands immediate measures such as transfusions Premature treatment may be detrimental to the patient The use of complex preparations that contain iron vitamin B_{12} folic acid and other substances under the impression that such therapy will afford adequate treatment for all types of anemia may confuse the interpretation of the diagnostic studies without correcting the anemia Even more important is the fact that treatment may produce a false sense of security when it corrects the anemia which is but one aspect of the disorder without affecting other more serious aspects of the disease When no diagnosis has been established often the best course is to withhold treatment until the

chronic microcytic anemia or it may occur during the stage of incomplete recovery.[8] In patients with this type of anemia the history often suggests iron deficiency, but the demonstration of a low level of serum iron and/or an increased iron binding capacity or the demonstration of reduced iron stores by examination of an appropriately stained specimen of marrow, or even a therapeutic trial with iron, may be necessary to establish the diagnosis.

SIMPLE MICROCYTIC ANEMIA

Simple microcytic anemia (MCV < 80 c μ, MCHC > 30%) is most often seen in association with various chronic diseases, circumstances where a normocytic anemia is more common.

HYPOCHROMIC MICROCYTIC ANEMIA

The finding of a hypochromic microcytic type of anemia (MCV < 80 c μ, MCHC < 30%) almost invariably means iron deficiency. The erythrocytes on a stained blood smear have an increase in central pallor and show considerable poikilocytosis and anisocytosis. Elongated, cigar forms, racquet cells, and microcytes are often numerous. The leukocytes and platelets are usually normal. The cellularity of the bone marrow may be subnormal, normal or increased; erythropoiesis is normoblastic in type. The existence of iron deficiency can be established definitely by finding a subnormal level of the serum iron associated with an increase in the latent iron binding capacity of the serum, or by the demonstration of reduced iron stores in the bone marrow.[8]

The other diseases that may be confused with iron deficiency occur much less commonly and usually can be distinguished without difficulty. The rare anemia that is due to pyridoxine deficiency is hypochromic and microcytic but the serum iron level is elevated rather than reduced.[48] In thalassemia the erythrocytes are hypochromic and microcytic, but the condition can generally be distinguished from iron deficiency by the family history, reticulocytosis, marked splenomegaly, the presence of alkali resistant hemoglobin, the elevated level of the serum iron and, if necessary, by the failure to respond to therapy with iron preparations. When the serum iron level is low in thalassemia minor, as has been demonstrated in some cases, the differential diagnosis may be difficult and examination of the sternal marrow for stainable iron may be helpful.[8]

When anemia is due to iron deficiency, it is important to determine the cause. Except in infants and young children a dietary lack should not be accepted as an explanation of iron deficiency unless there are modifying factors. Among these are the increased need of iron during pregnancy and growth. However, iron deficiency may develop despite an adequate diet if absorption is impaired because of a malabsorption disorder or partial gastrectomy. In an adult male, unless there is some absorptive defect, iron deficiency anemia can be considered as practically synonymous with chronic

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nature of the anemia is better understood It is rare that a delay in treatment until the necessary diagnostic studies are performed works to the disadvantage of the patient

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CHAPTER IV

Clinical Syndromes Associated with Anemia

PERNICIOUS ANEMIA

Synonyms Primary Anemia, Addison's Anemia, Biermer's Anemia

PERNICIOUS ANEMIA is a disease that has intrigued numerous investigators for many years The history of pernicious anemia is an "illustrative case of the development and progress of medicine First centuries of almost complete ignorance then the slow accumulation of clinical information from many scattered sources over a period of about a hundred years a period which was followed by the rapid progress in the past few decades The disease affords an example of the successful combining of information gained from animal experiments with careful clinical observations to discover the clinical cure of a previously fatal disease It is also an illustration of the way in which a significant contribution to clinical medicine stimulates further more basic investigation This in turn leads to a better understanding of human physiology and chemistry that has a significance far beyond a particular disease

HISTORY

The history of pernicious anemia has been reviewed by Haden[81] Credit for reporting the first case of pernicious anemia is generally given to Combe for his report to the Medico Chirurgical Society of Edinburgh on May 1 1822 Thomas Addison of Guy s Hospital is given credit for recognizing the disease as a clinical entity Addison described a progressive fatal type of anemia of unknown cause that occurred in both sexes generally beyond the middle period of life He observed the insidious onset the increasing weakness and pallor and commented on the remarkable absence of wasting despite the fatal outcome of the extremely debilitating disease[15] Biermer in 1871 independently described 15 cases of severe anemia to which he gave the name progressive pernicious anemia Interest in the disease was stimulated and numerous papers followed Ehrlich described the megaloblasts which he considered diagnostic of pernicious anemia[8] and provided a subject of interest and controversy for those interested in morphology that has continued to the present time The importance of the gastric secretion of acid in various diseases was studied by Cahn and von Mering in 1886 and in 1921 Levine and Ladd demonstrated the almost constant occurrence of achlorhydria in pernicious anemia in a study of 107 patients with the disease The relation of the gastric defect to the causation of pernicious anemia was demonstrated by Castle in 1928[4 5]

The studies of Whipple and Robscheit Robbins demonstrated the importance of food factors particularly liver in correction of posthemorrhagic anemia in dogs and suggested that such therapy deserved serious consideration in other more complex anemias such as pernicious anemia [9] Minot and Murphy s dramatic report on "The Treatment of Pernicious Anemia by a Special Diet followed soon after[18] The excellent results obtained with liver in this series of 45 patients with pernicious anemia were soon confirmed The significance of this contribution has been emphasized by Haden There is nothing more dramatic in medicine than the effect of liver therapy on a patient with pernicious anemia Only the use of sulfa drugs and other antibiotics such as penicillin afford such brilliant results "[80]

The use of whole liver by mouth led to the development of liver extracts of increasing potency that could be administered parenterally The search for the active principle of liver extract culminated in the discovery of vitamin B_{12} by Rickes and colleagues in 1948[21] Subsequent investigations by numerous workers have demonstrated important roles of vitamin B_{12} and folic acid in nucleic acid metabolism in the body

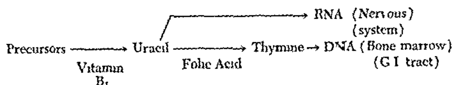
MECHANISM OF VITAMIN B_{12} DEFICIENCY IN PERNICIOUS ANEMIA

The relationship between the abnormal gastric secretion and the effectiveness of the liver diet in pernicious anemia was shown by Castle and Townsend[5] who utilized beef muscle normal gastric juice hydrochloric

acid and gastric juice from patients with pernicious anemia in their investigation. In a series of classic experiments they studied the hematologic effects of various combinations of these substances on patients with pernicious anemia in relapse. They demonstrated that patients with pernicious anemia lack a substance in the gastric juice that is necessary for the absorption of some factor present in meat that is essential for normal erythropoiesis. It was shown that this gastric factor, which could be destroyed by heat, is not hydrochloric acid. These studies thus established that the primary defect in pernicious anemia is *the failure of the stomach to secrete a substance*, probably an enzyme, that is necessary for the absorption of a dietary element essential to normal hematopoiesis. As a result of these observations it was postulated that an "extrinsic factor" present in the beef muscle reacted with an "intrinsic factor" secreted in the normal gastric juice to form an "erythrocyte maturation factor," which was the active principle that was stored in the liver. After vitamin B_{12} was isolated, Castle and coworkers repeated these experiments but substituted vitamin B_{12} for the beef muscle.[1] The results demonstrated that vitamin B_{12} is both an "extrinsic factor" and an "erythrocyte maturation factor." The function of the intrinsic factor is that of making possible the absorption of vitamin B_{12} from the diet for utilization in the bone marrow and for storage in the liver.

The problem of the identity and method of action of the intrinsic factor has been reviewed recently.[6] Investigations have shown that in man the intrinsic factor is secreted only by the glands of the body of the stomach and that there is no secretion of this factor in the pyloric end of the stomach or in the small intestine, as is the case in swine. The intrinsic factor, which is water soluble but not non-dialyzable, is destroyed by heat or boiling and can be precipitated with ammonium sulfate. These characteristics indicate that it is a protein, probably an enzyme. Other studies have shown that it is a mucoprotein which is capable of binding vitamin B_{12}.[6] Several theories have been advanced to explain the action of the mucoprotein. It has been suggested that it functions by binding vitamin B_{12} and preventing the absorption of the vitamin by the bacteria in the intestine. This explanation appears unlikely because it has been demonstrated that patients with megaloblastic anemia associated with abnormalities of the intestine respond to antimicrobial therapy but not to added "intrinsic factor."[9] Another possibility, also considered unlikely, is that the intrinsic factor actually unites with the vitamin B_{12} in order to form an active product.[6] A different role is suggested by the concept that intrinsic factor acts on the intestinal mucosa rather than on vitamin B_{12} to facilitate the absorption by the mucosal cells.

Although both vitamin B_{12} and folic acid are known to be important in normal hematopoiesis, the role of these vitamins and their relationship to each other remain uncertain despite considerable study. An interesting hypothesis that would explain many of the observations has been presented by Nieweg et al.[19] According to this theory vitamin B_{12} and folic acid act at different steps in the metabolism of the nucleic acids. This theory is shown in the following diagram.



If vitamin B_{12} is necessary for the synthesis of both RNA (*ribonucleic acid*) and DNA (deoxyribonucleic acid) and folic acid is necessary only for the synthesis of DNA many of the clinical evidences of the deficiencies of these vitamins could be explained DNA which is an essential constituent of chromosomes is utilized by tissues such as the gastrointestinal tract and the bone marrow where cell division is active RNA which occurs in the cyto plasm and the nucleolus of the cell is not involved with the chromosomes or cell division and is most important in cells that have a large amount of cytoplasmic substance without very active cell division In such circum stances one would expect that the greatest demand for RNA would occur in the central nervous system of the adult where the nerve cells do not divide but are the site of active protein synthesis that is necessary to main tain the function of the axones This theory which postulates that folic acid is concerned mainly with DNA metabolism would explain how gastro intestinal and bone marrow lesions might occur without any neurologic disturbance as a result of a deficiency of folic acid It would also explain how a deficiency of vitamin B_{12} could cause neurologic disease as a result of RNA deficiency as well as changes in the bone marrow and gastrointesti nal tract as a result of DNA deficiency This theory would further account for the effectiveness of thymine in correcting the anemia in patients with combined folic acid and vitamin B_{12} deficiency and the ineffectiveness of uracil in a patient with megaloblastic anemia of pregnancy due to folic acid deficiency [19] The importance of these vitamins in the metabolism and growth of cells in many parts of the body is shown by the increased size and altered appearance of the nuclei in the gastric mucosal cells obtained by gastric lavage of patients with pernicious anemia [1]

If vitamin B_{12} absorption is impaired sufficiently from any cause a macrocytic anemia and the clinical picture of pernicious anemia will de velop Such a situation occurs after total gastrectomy if the patient survives the three or four year interval that is usually necessary to deplete the body stores of vitamin B_{12} A deficiency of vitamin B_{12} also occurs in certain patients with fistulae diverticula and other lesions of the small intestine even though achlorhydria is not present In these conditions the cause of the vitamin B_{12} deficiency is not clearly understood but it appears to be related in some way to the bacterial flora of the intestine since the ability to absorb vitamin B_{12} is restored by treatment with oral antibiotics but is not corrected by the administration of the intrinsic factor [9]

A disease with hematologic and neurologic manifestations identical to those in pernicious anemia occurs in some individuals who are infected with the fish tapeworm Diphyllobothrium latum [3] The disease is found mainly

in Finland Norway and Sweden Only about one in five or ten thousand carriers infected with the tapeworm develops anemia[3] The pathogenesis of *the disease is not fully understood but the tapeworm appears to compete* successfully for the available vitamin B_{12} or interfere with the absorption of vitamin B_{12} because elimination of the parasite without other therapy is followed by recovery

The occurrence of a macrocytic anemia with the hematologic characteristics of pernicious anemia has been reported in patients treated with anticonvulsant drugs[9a] Only a small percentage of the patients so treated develop anemia of this type and the mechanism has not been established

CLINICAL MANIFESTATIONS

Pernicious anemia affects individuals of either sex occurs most often after the age of forty and is rare before the age of thirty Although pernicious anemia has been considered rare in Negroes and in dark skinned white races[30] the incidence in Negroes in the United States approaches that in Caucasians[10]

The clinical picture of pernicious anemia is variable Patients with untreated *pernicious anemia usually complain of an insidious onset of* weakness anorexia pallor indigestion and numbness and tingling in the extremities In older persons dyspnea palpitation dizziness and angina of effort often develop because of the effect of anemia on the circulatory system Soreness of the tongue and diarrhea may be present Although seldom severe some loss of weight usually occurs in a series of patients seen by the authors the average weight loss before treatment was 25 pounds in Negroes and 15 pounds in Caucasians[10]

In some patients with pernicious anemia particularly since the advent of folic acid and the widespread use of multivitamin preparations the hematologic abnormalities are mild or absent when neurologic manifestations are prominent Subjective and objective neurologic findings have been reported in 80 per cent of patients and were severe enough to cause some degree of incapacity in about 50 per cent[4] The most common manifestations are paresthesias incoordination disturbance of motility impairment of position and vibratory sense and absence of reflexes The symptoms range from subjective numbness and tingling of the extremities in the mildest form to an unsteady gait difficulty in locomotion and difficulty in buttoning the clothes in the more severely affected patients In the most severe form of the disease the patient is unable to walk and voluntary control of the bladder and rectum is impaired or lost Some patients undergo marked personality changes associated with memory loss impaired judgment disorientation hallucinations and delusions

At times the diagnosis of pernicious anemia is suggested by aspects of the patient's appearance such as gray hair sallow skin slight scleral icterus and atrophy of the papillae of the tongue The spleen is usually enlarged in hematologic relapse and is often palpable In most patients neurologic ex

amination reveals abnormalities of the peripheral nerves and posterolateral columns that are most marked in the lower extremities The earliest signs are usually some loss of vibratory sense in the lower extremities and impairment of sensation to pin prick and touch that may be either spotty or of the "stocking" type With more severe changes the Romberg test is positive position sense is impaired and the deep tendon reflexes are lost at the ankles and knees Further progression of the disease in the spinal cord is associated with the development of hyperreflexia and the presence of pathologic reflexes such as an extensor plantar response Paraplegia and loss of sphincter control are late manifestations The neurologic dysfunctions in pernicious anemia have been classified as (1) cerebral (2) olfactory (3) peripheral nerve and posterior column of the spinal cord (4) peripheral nerve posterior and lateral columns of the spinal cord [4] The distinction between peripheral neuropathy posterior column disease and combined system degeneration is useful as an indication of the severity of the disease but classification in this way is not always easy For this reason some authors quantitate the extent of the process by grading the severity of all the objective changes as I II III or IV [2]

LABORATORY EXAMINATION

In nearly every patient with uncomplicated and untreated pernicious anemia macrocytic anemia occurs Erythrocyte counts range from less than a million to near normal values The MCV is nearly always over 100 cu microns and is often in the range of 120 to 130 cu microns The hemoglobin values are not reduced as much as the erythrocyte count and the MCH is usually increased (over 33 $\gamma\gamma$) The blood smear shows considerable anisocytosis and poikilocytosis of erythrocytes that appear to be well filled with hemoglobin If the anemia is severe stippled red cells and nucleated red cells of the megaloblastic series may be seen In the untreated patient the *reticulocyte count is either normal or subnormal*

Leukopenia and thrombocytopenia occur commonly in pernicious anemia Leukocyte counts of 3000 to 4000 per cu mm are common and levels of 1600 to 2000 per cu mm sometimes occur The leukopenia is due mainly to a reduction in the granulocytes Some of the granulocytes show abnormalities such as hypersegmentation and an increase in size these cells are called "macropolycytes (Fig 21) In patients with *severe anemia the platelets* often number less than 100 000 per cu mm

The gastric secretion in pernicious anemia is abnormal in several respects The total quantity of the gastric juice is *greatly reduced and achlorhydria* is an almost constant finding These abnormalities persist after treatment Because exceptions occur so rarely failure to secrete free hydrochloric acid after the injection of histamine subcutaneously can be *considered an* almost essential feature for the diagnosis of pernicious anemia

The bone marrow in relapse is usually hypercellular The characteristic feature is the occurrence of the megaloblastic type of erythropoiesis (Fig



Figure 21 A Peripheral blood and *B* bone marrow from a patient with untreated pernicious anemia The peripheral blood smear shows macrocytosis anisocytosis poikilocytosis and a macropolycyte The bone marrow shows the megaloblastic type of maturation

21) If anemia is severe promegaloblasts and basophilic megaloblasts are increased in number and mitotic figures are numerous The polychromatophilic megaloblasts which are the easiest cells to identify in this series persist even after the anemia in the peripheral blood has been abolished by

transfusion [8, 18] The erythrocyte precursors in the marrow are extremely sensitive to specific therapy and the parenteral administration of even a few micrograms of vitamin B_{12} or the ingestion of a few milligrams of folic acid is sufficient to change the megaloblastic picture in the marrow to a normoblastic one within a day or two In such circumstances the appearance of the bone marrow may not be diagnostic However the morphologic abnormalities in the marrow in pernicious anemia are not confined to cells of the erythrocytic series Deficiency of vitamin B_{12} or folic acid also leads to the production of giant metamyelocytes and multisegmented macropolycytes These abnormalities in the granulocytic series do not disappear as promptly as the megaloblasts do after specific therapy and their presence may be helpful in diagnosis when the patient is seen for the first time soon after specific therapy has been started

Other laboratory tests in the untreated patient generally reveal an increased level of the indirect reacting bilirubin in the plasma (1 0 1.5 mg) an elevation of the serum iron an increase in fecal urobilinogen excretion a decreased blood volume and an increase in the plasma lactic acid dehydrogenase (400 to 4000 units) A diminution of the plasma protein is not unusual

DIFFERENTIAL DIAGNOSIS

The diagnosis of pernicious anemia in the untreated patient is usually made without difficulty The presence of macrocytic anemia associated with histamine fast achlorhydria and a megaloblastic bone marrow practically establishes the diagnosis The diagnosis is proved when the parenteral administration of vitamin B_{12} produces the characteristic hematologic changes These are the conversion of the megaloblastic type of erythropoiesis to the normoblastic type within a day or two a rise in reticulocytes during the first week and a return of the hemoglobin and red count to normal levels in succeeding weeks The magnitude of the reticulocyte response depends on the degree of anemia and varies inversely with the erythrocyte count The maximum reticulocyte count (R) that is expected to follow the intramuscular injection of liver extract or vitamin B_{12} in adequate amounts can be calculated from the formula

$$R = \frac{82 - 22Eo}{1 + 0\,5Eo}$$

where R represents the reticulocyte count in per cent and Eo represents the initial erythrocyte count in million per cu mm [11] For example if the initial erythrocyte count is 1 5 mil per cu mm

$$R = \frac{82 - (22 \times 1\,5)}{1 + (0\,5 \times 1\,5)} = \frac{82 - 33}{1 + 0\,75} = \frac{49}{1\,75} = 28 \text{ per cent}$$

In our experience it is not uncommon for the maximum reticulocyte count to fall slightly short of the expected maximum even when the reticulocytes are counted twice a day (Fig 22)

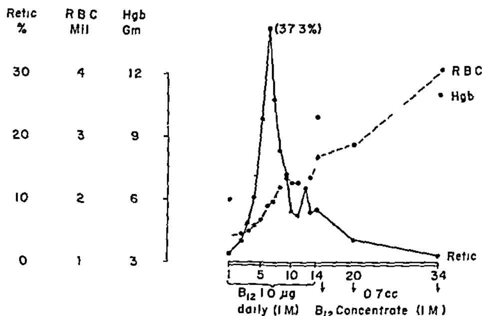


Figure 22 Reticulocyte response of a patient with untreated pernicious anemia to treatment with vitamin B_1 The maximum reticulocyte response which was reached about the end of the first week in this patient depends on the level of the erythrocytes before treatment

At times pernicious anemia must be differentiated from other conditions characterized by the megaloblastic type of erythropoiesis The most common disorders in this category are folic acid deficiency due to sprue and other dietary deficiencies and vitamin B_1 deficiency secondary to intestinal fistulae shunts and diverticula The fact that most conditions associated with folic acid deficiency do not produce histamine fast achlorhydria often helps to distinguish pernicious anemia from folic acid deficiency In addition the disorders associated with folic acid deficiency are often manifested by rather severe gastrointestinal disturbance accompanied by only minor neurologic symptoms while the reverse is more likely to be the case in pernicious anemia In doubtful cases the determination of the serum B_1 level or the use of some test of vitamin B_1 absorption such as the Schilling test may be necessary in order to establish the diagnosis [5]

The term "achrestic anemia" was proposed for the group of patients with a megaloblastic bone marrow and a peripheral blood picture identical with that of pernicious anemia who responded poorly or not at all to parenteral therapy with liver extract of known potency [1, 13] The patients that were reported had free HCl in the gastric secretion The morbid anatomy closely resembled that of pernicious anemia Known causes of macrocytic anemia such as dietary deficiency pregnancy sprue intestinal parasites and gastric operations were excluded in the reported cases Because extracts made from the livers of fatal cases were shown to give excellent hematopoietic

responses when administered parenterally to patients with pernicious anemia in relapse it was suggested that the disease in these patients was due to a failure to utilize or mobilize the antianemic principle that was present in the tissue stores [1] Sternal marrow biopsies showed various degrees of conversion from the megaloblastic to the normoblastic type of erythropoiesis after treatment with liver extract but usually resembled the marrow from an inadequately treated patient In our experience cases that fit this description are even rarer than the reported incidence of one per 100 patients with pernicious anemia [13] The report of Davidson suggests that at least some patients whose symptoms fit the description of achrestic anemia may well represent instances of folic acid deficiency In a group of 375 patients with macrocytic anemia associated with a megaloblastic bone marrow 25 with unexplained megaloblastic anemia (15 with achlorhydria) were refractory to therapy with potent extract The 15 who were treated orally with folic acid or proteolyzed liver responded to the treatment

When the patient with pernicious anemia has the neurologic features of the disease without the characteristic hematologic abnormalities the diagnosis may be difficult In such circumstances usually the patient has received vitamin B_1 or folic acid in an amount that is sufficient to convert the megaloblastic type of erythropoiesis to the normoblastic type and raise the erythrocyte count to near normal levels If examination of the peripheral blood and bone marrow does not suffice for the diagnosis a gastric analysis should be done If free hydrochloric acid is secreted after the histamine injection the diagnosis of pernicious anemia can be discarded with considerable assurance If histamine fast achlorhydria is present the Schilling test for vitamin B_1 absorption or some other similar test should be performed since it will often clarify the diagnosis

The tests of vitamin B_1 absorption are also helpful in the evaluation of patients without neurologic disease who have received treatment with vitamin B_{12} or liver extract before the diagnosis was established By such means the diagnosis can be verified or corrected without waiting months or years after specific therapy is withheld to see whether hematologic relapse occurs

TREATMENT

Prior to the report of Minot and Murphy in 1926 on the efficacy of liver the treatment of pernicious anemia was unsatisfactory and the disease was invariably fatal [18] Since that time more efficient preparations have been developed but all are forms of substitution therapy that must be continued for the life of the patient Vitamin B_1 is the essential factor and the parenteral administration of as little as 1 microgram each day to a patient in relapse is sufficient to restore the blood to normal and prevent the development of neurologic lesions if these are not already present In practice it is customary to administer larger amounts at longer intervals and to err toward an excessive dose rather than risk an amount that may be suboptimal

Although the selection of the details of the schedule may be somewhat

arbitrary some definite program of treatment should be followed The following schedule has been found satisfactory for patients in relapse in our experience vitamin B_{12} 30 micrograms intramuscularly three times during the first week then 30 micrograms once a week until the blood returns to normal This schedule produces the expected reticulocyte response within the first week or ten days and a return of the erythrocyte level to normal within six to ten weeks Thereafter the patient receives injections of 30 micrograms every four weeks or 60 micrograms every two months This program has maintained patients in hematologic remission and prevented the development of neurologic lesions However in one reported series of 51 patients treated with 50 micrograms of vitamin B_{12} parenterally each month definite neurologic relapse occurred in the absence of hematologic relapse three of these patients improved when the amount of vitamin B_{12} was increased and one responded to liver extract [6] Such reports appear to confirm the clinical impression that not all patients with pernicious anemia require the same maintenance dosage For this reason it is advisable to check the hematocrit levels every few months and the neurologic status at regular intervals so that the dosage of vitamin B_{12} can be adjusted if the therapy is not adequate Although effective oral preparations are available parenteral therapy is preferred because of greater reliability less expense and better supervision of the patient Unless careful supervision is maintained some patients will develop hematologic or more important neurologic relapse as a consequence of inadequate therapy In one report of 41 patients treated orally with vitamin B_{12} and folic acid 5 failed to return and in 14 others oral therapy was discontinued because of neurologic or hematologic relapse [6]

When an elderly patient is severely anemic the circulation is sometimes compromised by the low levels of the circulating hemoglobin When this occurs transfusions may be administered if it is considered inadvisable to wait for the erythropoietic effect of the vitamin B_{12} The use of packed cells is preferred to whole blood because the former gives the desired increase in circulating hemoglobin without as great an increase in blood volume

When severe neurologic complications are present vitamin B_{12} is often administered in amounts larger than those that are usually given to the patient with uncomplicated anemia This practice is based on clinical observations which led to the belief that the optimal therapeutic dose of vitamin B_{12} may not be the same for both the hematopoietic and nervous systems It has been recognized that there is little or no correlation between the severity of the anemia and the extent of the neurologic lesion because some patients present with severe anemia and little or no neurologic involvement while others have severe neurologic disease and only mild anemia Although neurologic complications rarely if ever develop if a normal hematologic status is maintained with parenteral vitamin B_{12} it has not been established that one microgram a day is the optimal initial treatment for the patient with existing neurologic involvement of significant degree The neurologic response to therapy is much slower than the hematologic and improvement may continue for several months after the blood has returned

to normal Some patients who show little neurologic improvement during the first month or two of therapy show gratifying improvement between the third and sixth month Apparently little or no improvement can be anticipated after ten to twelve months of therapy [1]

Because of these considerations we employ a larger dose of vitamin B_{12} in patients with neurologic involvement (100 micrograms three times a week for three months then 100 micrograms weekly for three months) although it is recognized that the value of a dose larger than that necessary to produce hematologic remission has not been established Although parenteral vitamin B_{12} is the most important treatment for patients with neurologic disease physiotherapy is often very helpful in patients who are severely affected It has been shown clearly that while folic acid therapy may maintain the patient in a normal hematologic status it does not prevent the development of neurologic lesions [3]

Vitamin B_{12} appears to be a complete therapy in pernicious anemia Dilute hydrochloric acid folic acid and liver extract are unnecessary Likewise iron is not indicated unless there is an associated iron deficiency from some other cause

Failure to respond to therapy or the occurrence of a hematologic relapse while the patient is receiving adequate amounts of vitamin B_{12} should raise the suspicion of the development of some infection renal insufficiency gastrointestinal bleeding or the appearance of a carcinoma of the stomach In former years the presence on the market of ineffective liver preparations was responsible for some therapeutic failures

COURSE AND PROGNOSIS

If the patient does not have significant neurologic involvement when he is first seen the prognosis is excellent He should be restored to his previous status and with proper maintenance therapy his outlook should be that of his age group with the possible exception of a greater likelihood of developing carcinoma of the stomach The reported incidence of carcinoma of the stomach in patients with pernicious anemia varies considerably One report placed the incidence in living patients at 1 7 per cent [9] while another roentgen study of 211 patients found gastric carcinoma in 8 per cent and benign polyps in 7 1 per cent Several reports place the frequency of carcinoma of the stomach at autopsy at from 8 4 per cent to 12 3 per cent [14, 31] figures that are three to four times the incidence in individuals of the same age group who do not have pernicious anemia [31] The increased frequency of carcinoma suggests the desirability of periodic examinations in an effort to detect the carcinoma in an early stage It has been recommended that the stool be examined for occult blood each month and that gastric cytology be studied every three to six months Suspicious cases should have gastroscopy and roentgenographic study of the upper gastrointestinal tract [31]

If neurologic complications are present the prognosis is related to the extent and duration of the manifestations If symptoms have been present

arbitrary some definite program of treatment should be followed The following schedule has been found satisfactory for patients in relapse in our experience vitamin B_{12} 30 micrograms intramuscularly three times during the first week then 30 micrograms once a week until the blood returns to normal This schedule produces the expected reticulocyte response within the first week or ten days and a return of the erythrocyte level to normal within six to ten weeks Thereafter the patient receives injections of 30 micrograms every four weeks or 60 micrograms every two months This program has maintained patients in hematologic remission and prevented the development of neurologic lesions However in one reported series of 51 patients treated with 30 micrograms of vitamin B_{12} parenterally each month definite neurologic relapse occurred in the absence of hematologic relapse three of these patients improved when the amount of vitamin B_{12} was increased and one responded to liver extract [6] Such reports appear to confirm the clinical impression that not all patients with pernicious anemia require the same maintenance dosage For this reason it is advisable to check the hematocrit levels every few months and the neurologic status at regular intervals so that the dosage of vitamin B_{12} can be adjusted if the therapy is not adequate Although effective oral preparations are available parenteral therapy is preferred because of greater reliability less expense and better supervision of the patient Unless careful supervision is maintained some patients will develop hematologic or more important neurologic relapse as a consequence of inadequate therapy In one report of 41 patients treated orally with vitamin B_{12} and folic acid 5 failed to return and in 14 others oral therapy was discontinued because of neurologic or hematologic relapse [6]

When an elderly patient is severely anemic the circulation is sometimes compromised by the low levels of the circulating hemoglobin When this occurs transfusions may be administered if it is considered inadvisable to wait for the erythropoietic effect of the vitamin B_{12} The use of packed cells is preferred to whole blood because the former gives the desired increase in circulating hemoglobin without as great an increase in blood volume

When severe neurologic complications are present vitamin B_{12} is often administered in amounts larger than those that are usually given to the patient with uncomplicated anemia This practice is based on clinical observations which led to the belief that the optimal therapeutic dose of vitamin B_{12} may not be the same for both the hematopoietic and nervous systems It has been recognized that there is little or no correlation between the severity of the anemia and the extent of the neurologic lesion because some patients present with severe anemia and little or no neurologic involvement while others have severe neurologic disease and only mild anemia Although neurologic complications rarely if ever develop if a normal hematologic status is maintained with parenteral vitamin B_{12} it has not been established that one microgram a day is the optimal initial treatment for the patient with existing neurologic involvement of significant degree The neurologic response to therapy is much slower than the hematologic and improvement may continue for several months after the blood has returned

to normal Some patients who show little neurologic improvement during the first month or two of therapy show gratifying improvement between the third and sixth month Apparently little or no improvement can be anticipated after ten to twelve months of therapy [4]

Because of these considerations we employ a larger dose of vitamin B_{12} in patients with neurologic involvement (100 micrograms three times a week for three months then 100 micrograms weekly for three months) although it is recognized that the value of a dose larger than that necessary to produce hematologic remission has not been established Although parenteral vitamin B_{12} is the most important treatment for patients with neurologic disease physiotherapy is often very helpful in patients who are severely affected It has been shown clearly that while folic acid therapy may maintain the patient in a normal hematologic status it does not prevent the development of neurologic lesions" [3]

Vitamin B_{12} appears to be a complete therapy in pernicious anemia Dilute hydrochloric acid folic acid and liver extract are unnecessary Likewise iron is not indicated unless there is an associated iron deficiency from some other cause

Failure to respond to therapy or the occurrence of a hematologic relapse while the patient is receiving adequate amounts of vitamin B_{12} should raise the suspicion of the development of some infection renal insufficiency gastrointestinal bleeding or the appearance of a carcinoma of the stomach In former years the presence on the market of ineffective liver preparations was responsible for some therapeutic "failures"

COURSE AND PROGNOSIS

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If neurologic complications are present the prognosis is related to the extent and duration of the manifestations If symptoms have been present

for only a few months the outlook is excellent particularly if the individual is less than 50 years old "[4] Signs of an upper motor neurone lesion such as the Babinski sign usually persist despite treatment but sometimes disappear Absent deep tendon reflexes usually return Marked improvement may occur promptly and maximal improvement occurs within ten or twelve months " [4] If paraplegia and loss of sphincter control have developed the outlook for recovery is poor However many patients who are severely ataxic and unable to walk improve after months of treatment and eventually recover sufficiently to resume most of their previous activities even though subjective and objective neurologic abnormalities persist

ILLUSTRATIVE CASES

Case 1

A 56 year old white housewife was admitted to the hospital for the evaluation of progressive numbness and pain in her hands and feet of two years duration In the last few weeks before admission her gait had become unsteady She had been taking "vitamin pills" as a tonic for many months On an admission thirteen years previously she was found to have histamine fast achlorhydria and hypochromic anemia the anemia responded to treatment with iron

Physical Examination The patient was an obese white woman in no apparent distress Pertinent abnormalities were found only on the neurologic examination These were absent gag reflex moderate spastic paraparesis and increased tendon reflexes in the lower extremities abnormal Babinski signs bilaterally impairment of position sense in the hands and feet and absence of vibratory sense in the feet and legs

Laboratory Examination Admission blood studies disclosed the following hct 42 per cent hb 14 3 gm per 100 ml rbc 4 3 mil per cu mm There was moderate anisocytosis of the red cells both microcytic and macrocytic forms were present Examination of the urine stool and spinal fluid was normal Gastric analysis on two occasions revealed the absence of free hydrochloric acid after the injection of histamine Examination of the bone marrow showed a normoblastic type of erythropoiesis A Schilling test for radioactive vitamin B_{12} (B_{12} Co^{60}) absorption was performed No significant absorption of vitamin B_{12} occurred (0% excretion) When the test was repeated with the addition of intrinsic factor absorption of the orally administered vitamin B_{12} occurred (9% excretion)

Diagnosis and Treatment A diagnosis of pernicious anemia with subacute combined degeneration of the spinal cord was made The patient was treated with vitamin B_{12} intramuscularly During the next ten months her gait improved gradually and returned to normal Impaired vibratory sense and paresthesias persisted

Comment The neurologic manifestations of pernicious anemia occurred in this patient in the absence of anemia The diagnosis was suggested by the

commonly in idiopathic steatorrhea [34] The relative importance of the different mechanisms that are responsible for the deficiency varies in different patients Poor absorption is usually the main factor but in some patients deficient diet or failure to secrete the gastric intrinsic factor is important [3]

If the absorption of vitamin B_1 or folic acid is impaired sufficiently a hematologic picture identical with that seen in untreated pernicious anemia occurs The anemia is macrocytic the erythrocytes manifest considerable anisocytosis and poikilocytosis and examination of the bone marrow reveals a megaloblastic type of erythropoiesis associated with the abnormal leukocytes that occur in pernicious anemia When a megaloblastic anemia which is the commonest type of anemia found in sprue [4] occurs in patients with the malabsorption syndrome a deficiency of folic acid is usually responsible The administration of folic acid orally or intramuscularly in doses of 10 to 30 mg per day usually produces a good hematologic response that is followed by marked clinical improvement [34, 37, 4] Some patients are refractory to treatment with vitamin B_1 parenterally [37, 4] while others respond at least partially [41] The response in the individual patient probably depends on the state of the vitamin B_1 stores and the degree of folic acid deficiency When both substances are deficient both folic acid and parenteral vitamin B_1 are necessary In some patients the gastrointestinal symptoms persist or recur despite improvement in the blood after administration of both folic acid and vitamin B_1

Iron deficiency anemia also develops in some patients with this syndrome as a result of blood loss from the gastrointestinal tract or because of impaired absorption of iron from the alimentary tract When iron deficiency is severe the anemia is hypochromic and microcytic but in milder degrees of iron deficiency the anemia is normocytic microcytic or hypochromic Anemia of this type responds to iron administrated orally in the usual dosage but in some patients parenteral administration is necessary A combined deficiency of iron and folic acid or iron and vitamin B^1 may result in a normocytic anemia that requires both iron and folic acid or iron and vitamin B_{12} in treatment [3]

Impaired absorption of vitamin B_{12} occurs in other conditions not associated with diarrhea or other evidence of the malabsorption syndrome Such impairment has been demonstrated in patients with blind intestinal loops fistulae strictures and diverticula of the small bowel [6] A severe deficiency may cause a megaloblastic anemia that responds to parenterally administered vitamin B_{12} but does not respond to the oral administration of both vitamin B_1 and intrinsic factor The improved absorption of vitamin B_1 that follows the administration of chlortetracycline and tetracycline suggests that in some way the abnormal intestinal flora inhibits absorption of the vitamin B_1 [36] Impaired vitamin B_1 absorption occurs regularly after total gastrectomy because of the absence of the gastric intrinsic factor [39] Vitamin B_1 deficiency rarely occurs after partial gastrectomy [38, 8] but sometimes develops after resection of the small bowel for regional enteritis or other similar disorders [40]

Comment This case illustrates many interesting points that are considered typical of pernicious anemia When first seen the patient was an elderly man with a severe anemia without significant weight loss The anemia was macrocytic and was associated with leukopenia, thrombocytopenia a megaloblastic bone marrow and histamine fast achlorhydria The diagnosis of pernicious anemia was established by the response to specific therapy Neurologic disease was never a problem After being well for nearly eight years the patient developed a carcinoma of the stomach which proved fatal in less than two years The incidence of carcinoma of the stomach in patients with pernicious anemia has been reported to be several times that in the general population The difficulty in dealing with the problem is shown by this patient Radiographic study of the upper gastrointestinal tract was performed every 12 to 18 months and yet the patient had an incurable carcinoma when it was recognized only one month after the onset of vague gastrointestinal complaints

ANEMIAS ASSOCIATED WITH DEFICIENT ABSORPTION FROM THE GASTROINTESTINAL TRACT (SPRUE AND OTHER SYNDROMES)

In the clinical entities characterized by malabsorption in the small bowel (sprue non tropical sprue idiopathic steatorrhea celiac disease lymphosarcoma and intestinal lipodystrophy) the most prominent symptoms usually are flatulence anorexia diarrhea bulky stools weight loss and glossitis Neurologic signs and symptoms other than tetany occur only in a minority of patients[4] Disturbed intestinal absorption is usually manifested by the presence of increased fat in the stools a flat oral *glucose tolerance curve* lowering of the serum calcium and roentgenographic abnormalities in the small bowel Although diarrhea occurs frequently in patients with these syndromes malabsorption also occurs in the absence of diarrhea

Various types of anemia develop as the result of the defective absorption of the factors essential for normal erythropoiesis In one group of 25 patients with idiopathic sprue 20 were found to have impaired absorption of vitamin B_1 that did not improve when intrinsic factor was administered[40] In another study lowered serum vitamin B_1 levels were found in about one third of the patients with steatorrhea[39] The absorption of vitamin B_1 in the presence of added intrinsic factor has been found to improve after the administration of corticotropin[3] Impaired absorption of folic acid has been shown to occur

some degree of iron deficiency antedates the pregnancy[44, 1] Severe iron deficiency is evidenced by the occurrence of a hypochromic microcytic type of anemia but a milder degree of iron deficiency may be associated with only minor changes in the indices and in the appearance of the erythrocytes[44, 47] When iron deficiency is present the serum iron level is reduced to less than 60 micrograms per 100 ml [47] and the latent iron binding capacity is increased erythropoiesis is normoblastic in type

Other types of anemia are much less common than that due to iron deficiency Macrocytic anemia attributed to dietary deficiency was observed in 8 4 to 25 7 per cent of the patients in one series[4] The higher incidence of this type of anemia occurred in a group of 70 patients of a distinctly low economic status Anemia associated with a megaloblastic type of erythropoiesis sometimes occurs during pregnancy or the puerperium. In one series of 45 patients with megaloblastic erythropoiesis indistinguishable from that seen in pernicious anemia the mean corpuscular volume was greater than 100 cu microns in only one half the patients The changes in the blood smears were similar to those seen in pernicious anemia but were less marked Free hydrochloric acid was present in the gastric secretion in three fourths of the patients Megaloblastic anemia is usually the result of a deficiency of folic acid but the factors responsible for its development are not apparent in all patients who develop this type of anemia Known dietary deficiencies vomiting and altered gastric secretion do not seem to afford adequate explanations and it has been suggested that multiple factors are concerned [3]

Recognition of the factors responsible for anemia during pregnancy is not always easy because iron deficiency occurs in association with normal indices[4] and megaloblastic anemia occurs with normal indices or even with microcytosis [3] Determination of the serum iron level which is reduced in iron deficiency and normal or elevated in other types of anemia and a study of the bone marrow usually enable one to establish the diagnosis Even with the benefit of the bone marrow examination recognition of the type of anemia may be difficult if the patient has taken multivitamin tablets These often contain amounts of folic acid that may be sufficient to convert a megaloblastic type of erythropoiesis to the normoblastic type and yet be inadequate to correct the anemia

When the patient with anemia is seen for the first time during pregnancy the possibility that the anemia may be the result of some coexisting disease rather than the pregnancy must be considered. Leukemia diseases of the lymphoma group renal disease or almost any other cause of anemia may be recognized for the first time during pregnancy The occurrence of pregnancy in a patient with one of the hemoglobinopathies may be serious because of the high incidence of complications in the mother or the child When one of the hemoglobinopathies is considered in the diagnosis it is important to differentiate the benign combination of a trait condition and an anemia of pregnancy from the more serious homozygous or doubly heterozygous abnormalities

ANEMIA ASSOCIATED WITH PREGNANCY

During normal pregnancy reductions in the hematocrit hemoglobin and erythrocyte count are found The maximal decrease occurs during the last trimester when the reduction may amount to 15 or 25 per cent[44, 46, 1] These changes are due at least in part to hemodilution since the plasma volume increases by 800 to 1300 ml while the red cell volume increases by only about 300 ml[43] Because of these disproportionate changes decreases in hematocrit, erythrocytes and hemoglobin levels can be considered to be physiologic to a certain extent

Not all changes that occur in the blood during pregnancy can be explained by hemodilution because the level of the serum iron falls[46, 47, 50] while the values for the free erythrocyte protoporphyrin[47, 0] and the latent iron binding capacity rise[46] In non pregnant patients elevations of the free erythrocyte proporphyrin and latent iron binding capacity are most often seen in iron deficiency More direct evidence of the frequent development of actual iron deficiency during pregnancy is the observation that the serum iron level fell in about 80 per cent of a group of patients who received no supplemental iron during pregnancy but remained normal in 80 per cent of those who received iron salts orally[47, 1]

The mother loses iron to the fetus and also with bleeding during delivery The iron requirements for the fetus which will be met at the expense of the mother amount to about 400 mg As the absence of menstruation during pregnancy allows the mother to conserve 325 mg of iron that would ordinarily be lost through menstruation the net added requirement of pregnancy is about 75 mg However the loss of iron in the placenta and in bleeding at the time of parturition has been estimated to average about 325 mg Calculations from these figures therefore indicate that the net additional iron necessary for pregnancy is 400 mg or more[47]

There is disagreement concerning the normal range of the blood values during pregnancy The following values have been said to represent the lower normal limits of hemoglobin and erythrocytes during pregnancy hemoglobin 10 gm per 100 ml erythrocytes 3 5 mil per cu mm[t] hemoglobin 11 3 to 11 5 gm per 100 ml red cells 3 7 mil per cu mm hematocrit 35 per cent[44, 50] The reported frequency of anemia in pregnancy varies from 30 per cent[42] to 80 per cent[1]

TYPES OF ANEMIA

Iron deficiency is the commonest cause of anemia during pregnancy It occurred in about 75 per cent of one series of patients[44, 51] In many instances

The anemia associated with chronic renal disease is nearly always normochromic and normocytic in type but a macrocytic anemia occurs occasionally. In one series of patients with uremia secondary to various types of renal disease the hemoglobin levels ranged from 5.6 to 8.4 gm per 100 ml and the erythrocytes counts ranged from 1.5 to 3.5 mil per cu mm[61] In the same series varying degrees of reticulocytosis 2.4 to 22.5 per cent occurred irregularly. The leukocyte and platelet counts are usually normal. Examination of the bone marrow reveals erythrocytic hypoplasia, normal cellularity or erythrocytic hyperplasia. The serum iron level and the unsaturated iron binding capacity are usually normal or slightly decreased unless significant blood loss has occurred. The antiglobulin (Coombs) test, hypotonic saline fragility test, mechanical fragility test, and serum bilirubin levels are usually normal[62] The fecal urobilinogen excretion is increased when hyperhemolysis is present.

For many years the anemia of uremia was ascribed to an accumulation in the blood of poisons secondary to defective renal function.[4] It was noted that whatever the nature of these "poisons" might be, the action on the marrow appeared to be selective because erythrocyte precursors seemed to be depressed while activity was normal or increased in the other marrow elements.[5] Histologic evidence of hypoplasia of the erythroid series was found on examination of the marrow only when the non protein nitrogen level of the blood was above 150 mg per 100 ml. Complete aplasia was not found and it was suggested that a defect in the delivery of red blood cells to the peripheral blood from the bone marrow might be a factor in the anemia.

In 1949 Emerson and Burroughs[7] published the first of a series of reports indicating that hemolysis contributes to the production of anemia in uremia in some circumstances. When the survival time of erythrocytes was measured by the Ashby technique of differential agglutination, it was found that normal donor cells were destroyed in the recipient uremic patient at one and a half to three times the normal rate. Erythrocytes from uremic patients survived normally in the normal recipient. It was concluded that some extracorpuscular factor present in the plasma of uremic patients was responsible for the premature destruction of red blood cells. Other investigators demonstrated that this hemolytic activity was not found in all patients and was not constant in the same patient.[62] The occurrence of hemolysis appeared to coincide with the periods of rapid progression of the underlying renal disease. *However it is apparent that hyperhemolysis is not the only mechanism* concerned in the anemia of uremia. Even during periods of increased hemolytic activity the decrease in the life span of the red blood cells is not sufficient to produce anemia if the marrow responds normally.[8] Thus while hemolysis contributes to the anemia in certain patients, it appears that depression of erythropoiesis is the most important and most common factor.

The diminished erythropoiesis in uremia has been demonstrated in several ways. It has been reported that in patients with uremia there is a decreased rate of utilization of radioactive iron[9, 63] and an increased storage of iron in the liver and spleen.[61] In vitro studies of bone marrow cells sus

TREATMENT AND PROGNOSIS

The best therapy for the anemia of pregnancy is preventive. In most patients iron deficiency anemia can be prevented by the ingestion of an adequate diet or by supplementation with 0.3 gm of ferrous sulfate three times a day. A single 0.3 gm tablet taken at bedtime is also effective. Most macrocytic anemias can be prevented by a liberal daily diet which contains one quart of milk, one fourth pound of lean meat, and one egg a day or by supplementing the diet with 5 mg of folic acid. If an iron deficiency anemia has developed it can generally be corrected by the administration of 0.3 gm of ferrous sulfate three times a day. In the occasional patient who does not tolerate oral iron because of gastrointestinal symptoms, parenteral iron may be required. Most patients with megaloblastic anemia do not respond to vitamin B_{12} or concentrated liver extract[43, 3] but usually respond to folic acid in a dose of 20 mg a day. The unusual occurrence of a response to the combination of vitamin B_{12} and ascorbic acid after the failure of each preparation singly has been reported.[49] As is true in other circumstances, the pregnant patient who is seriously ill with a severe degree of anemia may need blood transfusions before the diagnosis of the type of anemia can be established. Even in this situation it is rare that the necessary hematologic studies cannot be initiated or the necessary specimens obtained before the therapy is started.

The prognosis of the patients who develop anemia during pregnancy is excellent when proper treatment is given, if no serious complicating disorders are present. If iron deficiency develops, therapy with iron may be necessary for some months after the pregnancy in order to replenish the iron stores. Megaloblastic anemia rarely relapses after adequate therapy but may recur in subsequent pregnancies.[3]

ANEMIA ASSOCIATED WITH CHRONIC RENAL DISEASE

The majority of patients with chronic renal insufficiency develop a significant degree of anemia some time during the course of the disease. Anemia is not a common clinical feature of nephrosis but it occurs in nearly all patients with chronic renal insufficiency if uremia is present. The mechanism by which this anemia is produced has been the subject of a number of investigations but is still incompletely understood. The subject has been reviewed recently by Loge, Lange, and Moore.[63]

ing surgical removal of the thyroid gland[73] Since that time anemia has been recognized as a frequent development in patients with spontaneous myxedema and also in those who develop thyroid deficiency following surgery The incidence of anemia was reported as 52 per cent in one series of patients [7] and was found to be about 60 per cent in a series of 50 patients reviewed by the authors

MECHANISM OF THE ANEMIA

There is no evidence that the anemia in myxedema is due to blood loss and studies of the survival time of the erythrocytes in myxedema have revealed no evidence of hyperhemolysis [8] The anemia which results from diminished blood production appears to be an indirect effect of the lack of the thyroid hormone rather than being due to a deficiency of some specific factor necessary for erythropoiesis The anemia is of only moderate severity

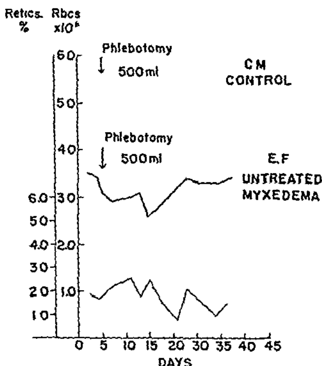


Figure 24 Response to bleeding of a normal patient and a patient with untreated myxedema The restoration of the erythrocyte count to the pre-phlebotomy level within the normal period of time indicates that the bone marrow of patients with untreated myxedema is capable of re ponding to a stimulus for erythropoiesis (From Leavell, Thorup and McClellan Tr Am Clin & Clim Assoc vol 68 1956)

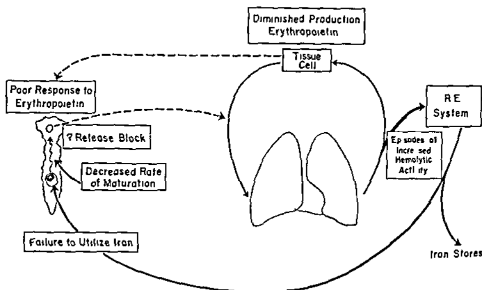


Figure 23 Schematic representation of the factors thought to be concerned in the production of anemia in chronic renal disease Despite the presence of anemia and the reduced oxygen carrying capacity of the blood there is a failure of the factors which normally operate to compensate for this deficiency

pended in culture media containing uremic serum have revealed a decrease in the rate of maturation of erythrocyte precursors[64] and a diminished rate of removal of iron from the culture media [66] The mechanism by which erythropoiesis is depressed in uremia is still uncertain It has been suggested that uremia in some way interferes with the production of erythropoietin [8] Jacobson et al concluded that the kidney is the site of production of erythropoietin or a precursor [60] but it is difficult to distinguish the hematologic effects produced by nephrectomy from those that are caused by azotemia Another aspect of this complex problem has been indicated by the studies of Rees et al which suggest that a disorganization of metabolism occurs in red blood cells that are suspended in uremic serum [6] The transfer of iron into the developing erythrocyte seems to be energy dependent and it is possible that a metabolic dysfunction which alters energy output interferes with iron uptake in vivo and in vitro

Figure 23 summarizes the various factors thought to play a role in the production of anemia in decompensated renal disease

ANEMIA ASSOCIATED WITH MYXEDEMA

The occurrence of anemia in myxedema was first noted by Charcot in 1881 [69] and in 1883 Kocher reported that anemia appeared gradually follow

to 99 μg per 100 ml (normal 60–150) Three of the patients with low serum iron levels were women who had mild normocytic anemia and the fourth was a man with a hematocrit of 40 per cent in whom no chronic blood loss could be demonstrated Whether these values indicate undetected blood loss or the poor absorption of food iron that has been reported to occur in myxedema is uncertain [80] Leukocyte counts varied from 3400 to 13 000 per cu mm but were usually normal The platelet counts were also normal Although bone marrow hypoplasia has been reported to be a feature of myxedema [6] bone marrow aspiration in this series revealed the cellularity of the marrow to be within normal limits in slightly more than one half the patients and hypocellular in the remainder Erythropoiesis was normoblastic in every instance

The results of the other laboratory tests are similar to those that are usually found in any patient with myxedema, such as a low basal metabolic rate elevation of the serum cholesterol decreased uptake of radioactive iodine and low plasma bound iodine The plasma bilirubin is normal although the plasma may appear slightly icteric because of carotinemia

DIAGNOSIS AND PROGNOSIS

If myxedema is considered in the differential diagnosis its presence can usually be established or excluded without difficulty by a consideration of the clinical features and by means of appropriate laboratory tests Myxedema is sometimes mistaken for pernicious anemia because the pallor and yellowish tint of the skin of patients with myxedema suggest pernicious anemia, as does the macrocytosis An incorrect diagnosis of aplastic anemia is also made at times because of the failure of the anemia to respond to treatment with iron vitamin B_{12} and other preparations

The anemia of myxedema generally responds to the administration of thyroid substance in the usual dosage (desiccated thyroid 0 03 to 0 1 gm per day) by a gradual rise in the hemoglobin and erythrocytes to normal levels in from three to nine months The prognosis is excellent as long as the patient continues treatment If there is an associated deficiency of iron or vitamin B_{12} the appropriate preparation must be given in addition to desiccated thyroid in order to restore the blood to normal

ILLUSTRATIVE CASE

A 42 year old white housewife was seen with the chief complaint of swelling of the eyelids During the preceding six or seven years she had noticed increasing fatigue aching in the arms legs and back and severe constipation She had noted marked intolerance to cold and stated that she "could wear a sweater even in the summer" During much of this time she had been treated unsuccessfully for "anemia" with various drugs

Physical examination revealed the face to be pale and puffy The upper eyelids were edematous The patient spoke slowly and her voice was hoarse

and responds but slowly to the administration of thyroid substance without the occurrence of reticulocytosis Bomford postulated that the anemia in myxedema represents an adjustment to the decreased metabolic needs of the patient and compared it to changes that occur when there is an excess of oxygen [68] The demonstration that thyroidectomized animals respond to bleeding and exposure to low atmospheric pressures by increased erythropoiesis indicates that the thyroid hormone per se is not necessary for increased erythropoiesis [71] [9] Evidence that a similar situation exists in man is afforded by the observation that patients with myxedema recover from bleeding without the administration of thyroid substance (Fig 21) [68] [4] The same conclusion can be drawn from the response of patients with myxedema and coexisting pernicious anemia or iron deficiency because the anemia responds at least partially to specific treatment with vitamin B_1 or iron without the administration of thyroid substance [68] Impaired absorption of either dietary iron or vitamin B_1 which have been demonstrated in myxedema [6 60] may be important in the production of anemia in some patients

CLINICAL MANIFESTATIONS

The onset of the symptoms in myxedema is often insidious with increasing weakness muscular aches lack of energy increased tolerance to heat and intolerance to cold Severe constipation is often a troublesome symptom Puffiness of the eyelids and swelling of the face and extremities are often apparent to the patient Contrary to the popular lay opinion weight gain is by no means a universal symptom in thyroid deficiency

When the patient is examined the diagnosis may be suggested by the appearance of the patient s face which is often pale puffy dull and expressionless The trunk and extremities frequently appear edematous but the edema does not pit on pressure Much of the hair normally present on the head and body may be lost and that which remains is coarse in texture In severe cases the skin is dry and scaly and has a pale yellow tinge The voice is usually hoarse and deep and speech is slow and deliberate In many patients the tongue is enlarged The recovery phase of the deep tendon reflexes is sluggish In addition to the signs characteristic of myxedema the patient may have the signs of associated cardiovascular disturbance such as edema ascites and pericardial effusion

LABORATORY EXAMINATIONS

The anemia is not severe in the uncomplicated case of myxedema The lowest erythrocyte count observed in the authors series of 50 patients was 3 0 mil per cu mm [5] The MCV ranged from 81 to 110 cu microns and was over 94 cu microns in 60 per cent The blood smears showed little or no poikilocytosis or anisocytosis There was no instance of hypochromic microcytic anemia in this group but the serum iron level was found reduced in four of ten patients in whom it was measured The values ranged from 32

of an illness characterized by menorrhagia retinal hemorrhages and marked pallor The peripheral blood which showed marked anemia and leukopenia was considered unusual in that no nucleated red cells were seen despite the severe anemia This apparent lack of increase in blood production in response to the anemia was explained by the fatty inactive appearance of the bone marrow at autopsy Following Ehrlich s description similar cases of this acute fatal illness were reported It was recognized also that a clinical picture identical with these idiopathic" cases occurred as a result of exposure to toxic agents such as benzene arsenicals and roentgen rays The concept of the disorder was broadened further by the inclusion of patients with the clinical picture and peripheral blood characteristic of "aplastic" anemia even though the bone marrow showed normal or increased cellularity[11] In addition cases have been considered to belong in this category if the anemia resulted from a failure of erythropoiesis when the anemia occurred alone or was associated with either neutropenia or thrombocytopenia

The syndrome of "aplastic anemia occurs in children as well as adults In 1927 Fanconi described three siblings with bone marrow hypoplasia pancytopenia and congenital anomalies[9] In a review of the 31 cases of Fanconi s syndrome reported up to 1955 small stature and hypogenitalism were found to be the most frequent of the twenty abnormalities that occurred Anomalies of the thumbs strabismus and renal defects were next in order Both hypoplastic and normally cellular marrows have been noted in patients thought to have the Fanconi syndrome[87] Other syndromes that have been described in infants are the familial hypoplastic anemia characterized by pancytopenia without associated congenital anomalies[91] and a type of hypoplastic anemia which affects the red cell series solely or predominantly[88]

MECHANISM OF THE ANEMIA

The primary defect in patients with this type of anemia is the failure of the bone marrow to produce erythrocytes in normal numbers in spite of adequate amounts of all the known hematopoietic factors The defect may be either an unresponsiveness of the erythropoietic tissues to adequate stimuli or the lack of some necessary stimulus[101] The presence of a defective response of the erythropoietic tissue is indicated by the reports of the presence of apparently adequate amounts of erythropoietin in the plasma of patients with hypoplastic anemia[94, 95] However it has been suggested that in children with congenital hypoplastic anemia there may be a congenital deficiency of erythropoietin because the infusion of "anemic plasma" (containing erythropoietin) into a patient of this type was followed by reticulocytosis[96] On the basis of the evidence available at present it appears that the defect in most patients is an inability of the erythropoietic tissue to respond to adequate stimuli

Causative Agents The defective erythropoiesis may be the result of

and deep The hair of the scalp was dry and axillary and forearm hair was scant The skin over the arms was dry and somewhat scaly The palms appeared yellow and the fingernails were soft and spoon shaped The remainder of the physical examination was within normal limits

Laboratory Examination Admission blood studies showed the following hb 11 3 gm per 100 ml hct 33 per cent rbc 3 0 mil per cu mm wbc 5000 per cu mm retics 0 6 per cent The 24 hour radioactive iodine uptake was 10 per cent (normal 12 to 42 per cent)

Treatment and Course A diagnosis of myxedema was made and the patient was advised to take desiccated thyroid 32 mg a day On this therapy there was marked symptomatic improvement and five months later the hematocrit was 37 per cent, hemoglobin 11 4 gm per 100 ml red count 3 8 mil per cu mm The dosage of the desiccated thyroid was increased to 64 mg per day Within another three months the patient was entirely asymptomatic the physical examination revealed no significant abnormalities but mild anemia persisted The hematocrit red count and hemoglobin values did not return to normal until nine months after treatment was started

Comment This patient illustrates most of the classic features of myxedema A diagnosis of myxedema was not made for some years because the presence of the disease was not suspected The history the abnormalities on physical examination and mild or moderately severe macrocytic anemia are all characteristic of myxedema As would be expected the anemia did not respond to injections of iron vitamin B_{12} and liver extract After the correct diagnosis was made the symptoms and objective signs of the disease disappeared gradually over a period of six months but the peripheral blood did not return to the normal range until nine months after treatment was started This slow response to specific treatment is characteristic of the anemia of myxedema

APLASTIC ANEMIA

Synonyms Refractory Anemia Chronic Bone Marrow Failure Hypoplastic Anemia Adynamic Anemia Aregenerative Anemia, Panmyelophthisis Aleukia Hemorrhagica Toxic Paralytic Anemia Hypoplastic Normocytic or Macrocytic Anemia due to Unknown Cause

Ehrlich described the first case of aplastic anemia in 1888 [90] This patient was a 21 year old white woman who died about one month after the onset

is the occurrence of a hereditary defect in the bone marrow that renders it more susceptible to either exogenous or endogenous toxins in later life[1] In some patients who present mainly or solely with chronic erythrocytic hypoplasia the presence of a thymoma has been considered to be a possible causative factor but at most tumors of this type can explain only a small proportion of the cases[81 106 113]

Hemolytic Aspects Although the main feature of this syndrome is deficient erythropoiesis a hemolytic element is also present in some patients who are usually included in this category This is shown by the demonstration of increased fecal urobilinogen excretion A decreased survival time of the erythrocytes has also been reported[114] When increased hemolysis is present in patients who have not been transfused the hemolytic element is of such minor degree that it would not result in anemia if the bone marrow were able to function in the normal manner Not infrequently increased hemolysis becomes a more important feature in patients who have been treated with numerous transfusions In this circumstance splenomegaly is often present

CLINICAL MANIFESTATIONS

Aplastic anemia may occur at any age In one series about half of the patients developed the disorder after the age of 50 years[11] Men were affected more often than women possibly as a result of a higher incidence of exposure to chemicals in occupations Aplastic anemia is rare in the Negro as compared to the white and this is especially true of the "idiopathic" type[10]

The onset of the disorder is generally gradual and is characterized by increasing weakness and other evidences of anemia such as exertional dyspnea palpitation and pallor Weight loss is infrequent In about one third of the patients abnormal bleeding is the initial complaint Although the onset is usually insidious in some patients the disorder begins abruptly with marked weakness fever other evidence of infection and hemorrhagic manifestations

On physical examination the most striking abnormality is the pallor of the skin and mucous membranes There is no yellowish tinge of the skin or sclerae Grayish brown pigmentation of the skin and testicular atrophy occur in an appreciable percentage of patients with this disease these abnormalities are found particularly in those who have been transfused and it seems likely that they are due at least in part to transfusion hemosiderosis In about one half of the patients there is evidence of abnormal bleeding in the skin mucous membranes or retinae at the time of the initial examination Although enlargement of the spleen liver and lymph nodes is unusual in the untreated patients these organs have been found to be enlarged in about one third of one group of patients who had been under observation and treatment for some time[102]

various forms of injury to the blood forming tissue In some patients the failure of erythropoiesis is clearly due to the effect on the bone marrow tissue of agents that are known to produce cellular damage if given in sufficient quantity Included in this category are x rays chemicals such as benzene and drugs such as nitrogen mustard TEM (triethylene melamine) urethane and the antimetabolites (antifolic compounds 6 mercaptopurine) Prolonged nitrous oxide anesthesia (five or six days) may also belong in this category [87, 89] In other circumstances the damage to the bone marrow occurs as a result of some individual idiosyncrasy to drugs or chemicals that are ordinarily innocuous Although it is possible that almost any drug or chemical may produce this reaction in a susceptible patient some drugs are more likely than others to be offenders In one review of 349 cases of aplastic anemia 137 were classed as idiopathic 80 were associated with the use of chloramphenicol alone or in association with other drugs and 132 followed exposure to other drugs chemicals or therapeutic irradiation [11] The following exposures to drugs or chemicals were found in these 132 patients (1) antibiotics (oxytetracycline chlortetracycline streptomycin) 27 cases (2) coal tar products (analgesics antipyretics salicylates) 22 cases (3) anticonvulsants 10 cases (4) antihistaminics 6 cases (5) barbiturates 16 cases (6) heavy metals (gold mercury bismuth) 4 cases (7) insecticides or weed killers 9 cases (8) organic solvents 15 cases 10 of which were exposed to benzol (9) sulfonamides 26 cases (10) miscellaneous associations (including nitroglycerin hair dye or rinse chemicals furniture wax or lacquer) 32 cases The authors emphasized that it cannot be stated categorically that any of the drugs actually caused the aplastic anemia that followed their use because of the possibility that the patients may have represented idiopathic cases of aplastic anemia in whom the association of the drugs was merely coincidental

Idiopathic Cases In a large percentage of patients with this syndrome no acceptable cause for the marrow failure can be found These cases are classified as "idiopathic Although the mechanism of the anemia in this group is obscure various possible explanations have been advanced Bomford and Rhoads suggested that erythropoiesis might be damaged as a result of the failure of the liver to inactivate completely hormones and other chemicals in the body with the result that a chemical compound toxic to the bone marrow might be produced by the abnormal metabolism of normal or innocuous compounds [8] Another possibility damage to the bone marrow by an auto immune process has been suggested as a result of the recognition of the role of antibodies in the production of hemolytic anemia and thrombocytopenic purpura [88, 93] Auto antibodies against erythrocytes leukocytes and platelets have been reported in a patient with aplastic anemia who had never been transfused [85] A possible etiologic relationship between leukemia and aplastic anemia must also be considered because leukemia sometimes develops in patients with aplastic anemia and both diseases have been reported to occur after exposure to benzol[53] or irradiation [98] Still another possibility which is suggested by the racial incidence and reports of a familial tendency

even though a definite diagnosis cannot be made If what is considered a satisfactory marrow sample is not obtained after needle aspiration at several different sites surgical trephining should be done to obtain a sample Although leukemia sometimes becomes manifest only months or years after the appearance of anemia, the distinction can be made in most patients after a relatively short period of observation In the 14 autopsied patients in one series the diagnosis of aplastic anemia was confirmed in all but one patient [16] In this patient, who had aplastic anemia while under observation for five years the presence of acute myeloblastic leukemia became manifest six weeks before death When leukemia appears some years after the onset of the anemia in a patient diagnosed as having aplastic anemia, a question as to the relationship of the two diagnoses arises The question is whether or not the patient has both aplastic anemia and leukemia, possibly from the same or related causative factors or only leukemia that has masqueraded as aplastic anemia

Aplastic anemia must be distinguished from pernicious anemia but this is usually not difficult because of the differences in appearance of the skin and the tongue and the absence of central nervous system involvement in aplastic anemia Most patients with aplastic anemia retain the ability to secrete hydrochloric acid and this finding is sufficient to practically exclude pernicious anemia from consideration Examination of the bone marrow even though hypocellular generally permits the identification of erythropoiesis as the normoblastic type rather than the megaloblastic type seen in untreated pernicious anemia Examination of the smear of peripheral blood also helps to distinguish between the two diseases even when the anemia is severe and macrocytic in aplastic anemia a smear of the peripheral blood usually shows but little poikilocytosis and anisocytosis

At times the problem of the differential diagnosis between the idiopathic aplastic anemia and anemia due to generalized carcinomatosis may arise The finding of young leukocytes and nucleated red cells in the peripheral blood suggests the possibility of carcinomatosis of the bone marrow The presence of metastases can be established sometimes by a bone marrow aspiration which reveals the malignant cells and at other times by radiographic study of the skeleton which discloses evidence of osseous involvement

TREATMENT

The treatment of this disorder is unsatisfactory in general but nevertheless the patient is treated as effectively as possible in the hope that recovery or remission will occur The most effective form of treatment is the removal of the cause For this reason patients should be questioned carefully about exposure to drugs or chemicals and should be removed from contact with any suspicious offenders

In most patients particularly with disease of the idiopathic type the most important treatment is blood transfusion Although the decision re

LABORATORY EXAMINATION

In a series of 50 patients studied at the University of Virginia Hospital the following peripheral blood conditions were noted pancytopenia 37 anemia alone 7 anemia with leukopenia 4 anemia and thrombocytopenia 2 [10] The anemia was macrocytic (MCV > 94 cu microns) in 60 per cent and normocytic in the remainder A mild reticulocytosis of 2 to 5 per cent occurred at some time during the course of the disease in 21 patients but an absolute increase in reticulocytes was rarely seen Only 9 patients had reticulocyte counts that were persistently 0 1 per cent or less In the same series of 50 patients 41 had leukopenia There was a relative lymphocytosis in 37 and an absolute lymphocytosis (over 3000 per cu mm) in 6 Only 5 had an absolute decrease in lymphocytes (less than 1500 per cu mm) The platelet counts varied from practically zero to normal levels but were markedly reduced in the majority of the patients

The marrow specimen obtained by aspiration or surgical biopsy usually shows decreased cellularity In the authors series the marrow was hypocellular in 74 per cent normal in 16 per cent and hypercellular in 10 per cent [10] Similar discrepancies between the cellularity and function of the bone marrow have been reported by others [6, 10, 11] In the bone marrow of many patients with this disease there is an increase in mononuclear cells that generally are classed as lymphocytes although their identity and origin are uncertain The differential count of the cells in the bone marrow often reveals increased percentages of plasma cells and reticulum cells

The results of other laboratory procedures are often interesting and sometimes are helpful in diagnosis The level of the serum iron may be increased and the latent iron binding capacity may be saturated even before the patient has received any blood transfusions [10, 107] The serum bilirubin level is usually normal but increased fecal urobilinogen excretion has been observed in some patients [8, 10] Liver function tests are usually normal unless the patient has received a large number of transfusions which lead to increased iron deposition in the liver [10]

DIFFERENTIAL DIAGNOSIS OF APLASTIC ANEMIA

The disease which causes the most difficulty in differential diagnosis is leukemia Even though careful studies of the blood and bone marrow do not reveal leukemia when the patient is first seen the possibility that the patient may still develop evidence of leukemia at a later date continues to be a source of concern The detection of significant enlargement of the spleen before the patient has been transfused should always raise the suspicion that the patient has leukemia or some other disease of the lymphoma group rather than aplastic anemia The distinction between leukemia and aplastic anemia can usually be made as a result of the bone marrow examination and a careful study of the peripheral blood Any significant increase in very young cells in the marrow or peripheral blood should raise the suspicion of leukemia

of their effect on the capillaries even when they produce no change in the cellular elements Prednisone is usually administered in a dose of 20 to 50 mg per day a dose larger than 50 mg may be advisable in acute hemorrhagic episodes but the effects are of short duration

Antibiotic drugs should be used when infection is present but their use as prophylactic agents appears inadvisable because of the emergence of resistant strains of bacteria and the development of fungus infections

Both thymectomy and splenectomy have been employed in treatment In some patients with thymoma and pure red cell anemia thymectomy has been followed by improvement in the anemia[81, 106, 113] Splenectomy has been effective in some patients with chronic erythrocytic hypoplasia and in some with pancytopenia Although there are exceptions[8] the experience of most clinics is that few patients respond well to splenectomy and for this reason the operation cannot be considered a standard method of treatment Splenectomy is most likely to be helpful if there is an associated hemolytic component[16, 103] Hyperhemolysis may be a feature of the disease initially but it is more likely to become prominent after the patient has received numerous transfusions If the patient requires a 500 ml blood transfusion oftener than every ten days and is not bleeding excessive hemolysis is probably present[1, 3] Good results from splenectomy have also been reported in patients who respond to corticoid therapy with a reticulocytosis[103]

CLINICAL COURSE AND PROGNOSIS

Although aplastic anemia must be considered a serious and generally fatal disorder the outlook is not as poor as is often thought Most of the earlier impressions were based on single case reports or on a series of 62 patients reported in 1919 in which only 3 patients survived more than a year[109] A more optimistic prognosis was indicated by another series of 66

Table 7 Prognosis in Aplastic Anemia

Survival in 50 Patients with Aplastic Anemia

Presenting Type of Peripheral Blood	No. of Pts	Alive: Years Survival 1	3	5	10	No. of Pts	Dead: Years Survival 1	3	5	10
Anemia	4	4	2	2	1	3	3	3	2	1
Anemia Leukopenia	0					4	4	3		
Anemia Thrombocytopenia	0					2	2	1	1	1
Pancytopenia	11	11	7	1		28	14	8	5	2
Total	15	15	9	3	1	35	23	15	8	4

These figures relate the survival time of patients with aplastic anemia to the abnormalities in the peripheral blood and demonstrate the more serious prognosis in pancytopenia (From Mohler and Leavell Ann Int Med vol 49 1958)

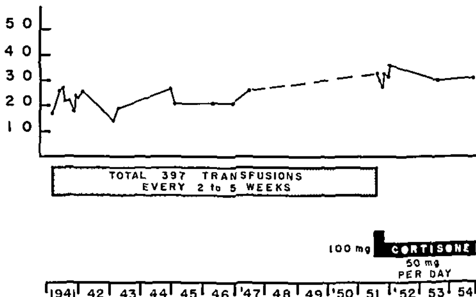


Figure 25 Chart showing response of a patient with chronic erythrocytic hypoplasia to treatment with cortisone Although this patient required an average of 400 ml of blood per week for ten and a half years no further transfusions were necessary after the institution of treatment with cortisone (From Mohler and Leavell Ann Int Med vol 49 1958)

garding the number and frequency of transfusions must be an individual one for each patient the hematocrit seldom need be raised to levels higher than 33 or 34 per cent If the blood is returned to the normal level whatever active erythropoiesis that is present may be depressed [69, 9] The vast majority of patients accommodate to a lower than normal level of hemoglobin without disagreeable symptoms Moore has recommended that the patient be given a 500 ml transfusion of blood less than five days old on three successive days when the hemoglobin level falls below 9 0 gm per 100 ml [103] Dangers of transfusion that restrict their usefulness are hemolytic transfusion reactions febrile reactions to the leukocytes in the transfusion [84] the danger of infectious hepatitis and the long term danger of exogenous hemochromatosis [16]

Most of the patients with aplastic anemia deserve a therapeutic trial with corticosteroids because some patients appear to respond to one or the other of these preparations [101, 11] A good hematologic response is more likely to occur if erythrocytic hypoplasia alone is present [109, 10, 110] Cobaltous chloride in a dose of 100 to 180 mg per day has also been reported to be effective in a few patients [111] Iron vitamin B_1 folic acid and thyroid substances are of no value

Hemorrhagic complications in aplastic anemia are usually due to thrombocytopenia Corticosteroids may be helpful in controlling bleeding because

Laboratory Examination Studies on admission disclosed the following hct 30 per cent hb 11 gm per 100 ml rbc 3 2 mil per cu mm wbc 3700 per cu mm smear segmented cells 4 per cent lymphocytes 96 per cent platelet count 16 000 per cu mm On the second day the hematocrit was 29 per cent wbc 1600 per cu mm (only 2 per cent segmented cells) the platelet count was 16 000 per cu mm The stool examination disclosed the presence of occult blood Several aspirations of the bone marrow revealed marked hypoplasia of all the marrow elements

Course A diagnosis of aplastic anemia was made and the patient was given prednisone 50 mg daily He continued asymptomatic for the first 48 hours On the third day after admission the temperature rose to 105 F Despite hourly doses of one million units of penicillin 2 0 gm of streptomycin and blood transfusions the patient developed peripheral vascular collapse and died on the same day Blood cultures were positive for Staph pyogenes and Staph aureus coagulase positive Death was attributed to septicemia

Post mortem Post mortem examination revealed vascular congestion of nearly all organs Bone marrow sections from ribs vertebrae and femur showed marked hypoplasia of the myeloid and erythroid series The spleen was congested and the lungs were emphysematous Sections of the liver revealed diffuse periportal necrosis with focal hemorrhage and some areas of regeneration changes that suggested toxic necrosis

Comment This patient with aplastic anemia had an acute illness manifested by pancytopenia and a hypocellular bone marrow Death was the result of a blood stream infection Idiosyncrasy to a drug chloramphenicol was suspected but as is usually the case the suspicion could not be confirmed or disproved because the patient had received many other drugs Although it is difficult to be sure of the role of any particular drug in cases of this type drugs that are known to cause blood dyscrasias even rarely should be used with caution and only when a safer drug will not suffice

Case 2

A white government official who had been admitted to the hospital several times between 1920 and 1950 because of hemorrhages from a duodenal ulcer was admitted in January 1956 at the age of 82 because of mild congestive heart failure and was found to have a normochromic normocytic type of anemia Admission blood studies showed the following hb 6 5 gm per 100 ml rbc 2 5 mil per cu mm hct 20 per cent wbc 5500 per cu mm retics 0 6 per cent differential count (per cent) segs 50 lymphs 37 monocytes 10 basophils 2 The platelets were normal in number The serum iron was 127 μg per 100 ml and the unsaturated iron binding protein was 185 μg per 100 ml Blood urea was normal The bone marrow obtained by aspiration appeared active and normoblastic No cause for the anemia was found and he was treated with blood transfusions

Treatment and Course The anemia recurred and failed to respond to

patients reported by Bomford and Rhoads in 1941 many of the cases were due to toxic exposure and complete recovery occurred in 15 [8] In a group of 50 patients seen by the authors 10 per cent had a complete remission and an additional 10 per cent had a partial or temporary remission of sufficient degree that transfusions were not necessary [10]- Such temporary remissions lasted as long as twelve years In a group of 35 patients who were followed until death the duration of the disease varied from five weeks to twenty years and averaged three years [10] (See Table 7)

The prognosis is best when the offending agent can be recognized and eliminated because the marrow may recover if it is not too severely damaged The outlook is better in patients with only erythrocytic hypoplasia than in those with pancytopenia because the complications of infection and hemorrhage are less common in the former group Patients with pure erythrocytic hypoplasia generally survive for more than a year and often live ten years or more about one third of the patients with pancytopenia die in the first year and the five year survival is probably about 20 per cent [10] In the authors experience the outlook has been better for patients with hypercellular marrows than for those with hypoplastic marrows but individual exceptions occur

The clinical course of the disease is extremely variable Some patients have an acute illness with septicemia fever prostration and bleeding The outlook in such patients is poor and death usually occurs within a matter of a few weeks or months Fortunately most patients have a longer and more benign course extending over a period of years When death occurs in patients with aplastic anemia it is usually the result of an infection such as septicemia or pneumonia that may be due at least in part to the leukopenia The next most common cause of death is external or intracranial hemorrhage which is related to the thrombocytopenia Other patients die from coexisting disorders such as vascular disease or from complications of therapy and a few develop leukemia as a terminal event

ILLUSTRATIVE CASES

Case 1

A 55 year old white laborer was treated for sixteen years for asthma emphysema and bronchiectasis During this time he had numerous acute respiratory infections with normal leukocyte responses and he received repeated courses of antibiotics During the two months prior to admission he had received three courses of chloramphenicol and also tetracycline penicillin and streptomycin for acute pulmonary infections Because of a petechial rash of four or five days duration he was admitted to the hospital

Physical examination disclosed that petechial hemorrhages were present on the trunk, extremities palate and conjunctivae The liver and spleen were not palpable Except for the pulmonary changes due to emphysema and bronchiectasis the examination was otherwise essentially normal

this type is usually given to Heuck who in 1879 reported two cases that were considered to show the chance *association of myelogenous leukemia* and osteosclerosis[1 1 1 8 1 6] Ever since that time there has been considerable confusion and controversy concerning terminology classification and causation of the syndrome Several thoughtful and comprehensive reviews of the clinical or pathologic aspects of the problem that also contain extensive bibliographies have appeared in recent years[1 0 1 1 1 1 8 136] Although some authors differ as to what should be classified as "primary and "secondary types of this syndrome some such grouping is desirable for a better understanding of the problem The clinical manifestations of this syndrome occur in patients known to have some primary disease such as carcinomatosis and also in patients in whom no primary disease can be demonstrated *The latter group is usually diagnosed myelofibrosis" or* agnogenic myeloid metaplasia "

SECONDARY OR SYMPTOMATIC MYELOID METAPLASIA

The clinical features of this syndrome splenomegaly anemia young cells in the peripheral blood and extramedullary hematopoiesis have been reported in a number of diseases that are sometimes classed as metaplasia myelophthisic anemia and space consuming lesions of the bone marrow The following diseases have been found to be associated with the syndrome carcinomatosis tuberculosis multiple myeloma Hodgkin s disease septicemia Gaucher s disease leukemia neurofibromatosis polycythemia vera[110 123 130 133] The simple occurrence of nucleated red cells in the peripheral blood without other evidence of extramedullary hematopoiesis is not sufficient for the inclusion of a patient in this syndrome Nucleated red cells have been found in the circulating blood in a number of disorders They are seen most often in association with hemorrhage pernicious anemia hemolytic anemia leukemia and carcinoma but also occur in other disorders such as heart failure severe infection and cerebrovascular accidents[134]

PRIMARY OR AGNOGENIC MYELOID METAPLASIA MYELOSCLEROSIS MYELOFIBROSIS

Most of the controversy regarding patients in this category has centered around the nature of the underlying process It has been considered to be (1) leukemic in nature (2) a proliferative or neoplastic disorder closely related to but different from myelocytic leukemia and polycythemia (3) a *response of the marrow and reticuloendothelial tissue to injury of some sort* According to one review 9 authors considered the disorder to be leukemic 29 believed it to be reactive but not neoplastic and 12 were undecided[136]

The evidence for the leukemic nature of the process has been reviewed by Heller Lewison, and Palin[1 1] These authors studied two autopsied cases and one splenectomized patient the bone marrow of both of the autopsied cases showed intense myeloid hyperplasia and only one showed small widely

oral iron folic acid vitamin B_1 or cobaltous chloride In July 1956 examination of the bone marrow disclosed almost complete absence of erythropoiesis and slightly depressed myelopoiesis During the period from January to July he received transfusions amounting to an average of 1500 ml of whole blood every month. In August 1956 when the rbc was 1 4 mil. per cu mm hb 3 9 gm per 100 ml hct 11 per cent retic 0 per cent prednisone was started in a dose of 20 mg daily Striking improvement occurred in the anemia and the bone marrow reverted toward normal. On a maintenance dose of 10 to 15 mg of prednisone a day the hematocrit remained in the range of 38 to 41 per cent for six and one half months without transfusions

Unfortunately an exacerbation of a previously mild diabetes occurred and the symptoms of peripheral neuritis became so severe that prednisone was discontinued in February 1957 The neurologic symptoms subsided but on April 9 1957 the hematocrit had fallen to 16 per cent Subsequently the anemia failed to respond to prednisone testosterone or cobalt and transfusions have been required regularly during the past two years The leukocyte and platelet counts remain normal while the reticulocyte count is usually zero Extensive studies have failed to reveal any apparent cause for this anemia

Comment This elderly clerical worker has aplastic anemia of the *chronic erythrocytic hypoplasia type* This syndrome is characterized by anemia with almost complete absence of reticulocytes and erythrocytic hypoplasia in the bone marrow the levels of the leukocytes and platelets in the circulating blood are normal Anemia of this type generally pursues a chronic course refractory to all therapy except transfusions but in some patients it responds to treatment with prednisone thymectomy or other measures The symptoms generally are due to anemia bleeding and infections are rarely serious problems

MYELOID METAPLASIA

Synonyms One review lists twenty five different names that have been suggested for this syndrome[1 1] Those that are used most commonly are agnogenic myeloid metaplasia myelofibrosis chronic non leukemic myelosis aleukemic myelosis leukanemia leukoerythroblastic anemia myelosclerosis osteosclerosis

This syndrome is characterized by splenomegaly anemia the presence of nucleated red blood cells and immature granulocytes in the circulating blood and the occurrence of extramedullary hematopoiesis in the liver and spleen There is considerable variation in the histological appearance of the bone marrow in the reported cases Credit for the first report of a case of

The most significant feature of the physical examination is the spleno megaly Palpable enlargement of the spleen is almost invariably present and is sometimes so extreme that the firm spleen fills the entire left side of the abdomen and extends into the right lower quadrant Hepatomegaly generally occurs but is usually less prominent than the splenomegaly Significant en largement of the superficial lymph nodes is exceedingly rare Not infre quently a bleeding tendency is manifested by ecchymoses and petechiae or retinal hemorrhages Pallor of the skin and mucous membranes is usually evident and jaundice of mild degree is sometimes apparent in the skin and *sclerae* The other abnormalities found on the physical examination are those that occur commonly in older persons with a chronic disease associated with anemia, such as evidence of loss of weight cardiac enlargement and pre cordial systolic murmurs

LABORATORY EXAMINATION

The most *significant hematologic feature* of this disorder is an anemia that is characterized by marked anisocytosis and poikilocytosis associated with the occurrence of nucleated red cells and myelocytes or myeloblasts in the peripheral blood The degree of anemia the levels of leukocytes and platelets and the numbers of immature erythrocytes and granulocytes vary within a wide range Patients with normal or polycythemic blood levels sometimes are also included as examples of this syndrome if immature cells *are present in the circulating* blood [1, 136]

In Linman and Bethells series of 56 patients the hemoglobin ranged from 5 0 to 14 0 gm per 100 ml and was less than 9 0 gm per 100 ml in 21 [1, 8] The mean corpuscular volume varied from 71 to 103 cu microns and was normal in 60 per cent of the subjects The mean corpuscular hemoglobin was normal in 41 patients and low in 15 Examination of the blood smear often suggests the diagnosis because of the presence of a marked degree of poikilocytosis—particularly "teardrop forms" and elongated cells—*anisocyto sis* and the presence of nucleated red cells The nucleated red cells are usually few in number but occasionally they outnumber the leukocytes [1, 7] Most often these cells are late normoblasts but basophilic and polychromato philic normoblasts and cells that resemble megaloblasts are sometimes seen The reticulocyte count is often normal but mild or moderate reticulocytosis of 3 to 5 per cent is common When hyperhemolysis is present the fecal uro bilinogen excretion is increased and the level of the serum bilirubin *is often* elevated

The leukocyte count is usually elevated[116, 1, 8] but a range of 1200 to 45 800 per cu mm was found in one series Myeloblasts progranulocytes and myelocytes are usually found in the peripheral blood It has been noted that the number and identity of the immature granulocytes in the peripheral blood represent a "shift to the left" rather than a leukemic hiatus"[1, 3] Marked diminution and even the disappearance of the immature leukocytes has been observed to follow a series of transfusions in several patients with

scattered areas of fibrosis but the authors emphasized that they considered their comments applicable to cases reported in the literature under the twenty five other names as well as the term aleukemic myelosis" which they used The authors who concluded that the syndrome is a form of myelogenous leukemia considered that the presence of fibrosis in the marrow osteosclerosis in the bones the absence of leukemic invasion in the organs and the appearance of the spleen were not adequate grounds for excluding leukemia

Wyatt and Sommers[136] studied 30 patients 20 of whom were autopsied and concluded that the disorder was non leukemic in nature It was thought that the primary lesion was a necrobiosis of the maturing hematopoietic cells which was followed by overgrowth of the marrow reticulum ossification and extramedullary hematopoiesis The study of the histories and a retrospective analysis for possible causative agents in these cases revealed a list of possible factors similar to those that are considered important in aplastic anemia This suggested that prolonged marrow exposure to certain substances might be an important factor A similar conclusion is suggested by another report of 6 patients with agnogenic myeloid metaplasia It was noted that two patients gave histories of heavy exposure to benzol two had daily exposure to paint removers one worked with carbon tetrachloride and one washed auto parts with high test gasoline [13]

Another hypothesis that has been advanced to explain the disorder considers that it is one manifestation of a proliferative disorder that involves the multipotential primitive mesenchymal cells According to this concept myelofibrosis myelogenous leukemia megakaryocytic myelosis and polycythemia vera are similar and related processes[118 1 1 9 135] Cases that show varying degrees of histologic evidence of these different disorders and cases that undergo transition from a typical example of one condition into a typical example of another are interpreted as evidence of the validity of this hypothesis

Although it is probable that the controversy as to the nature and causation of this syndrome will continue it appears that the concept of this syndrome as a proliferative disorder which does not exclude a reaction to toxic agents affords the best working hypothesis at the present time

CLINICAL MANIFESTATIONS

This rather uncommon disorder occurs in either sex Symptoms generally appear first after the age of 50 but sometimes begin before the age of 40[1 1 1 8 136] The onset is usually gradual and the patients complain of weakness and easy fatigue or of upper abdominal discomfort that is caused by the enlargement of the spleen and liver Weight loss of 15 to 20 pounds is common Aching in the extremities leg cramps and peripheral edema sometimes occur Cutaneous hemorrhages either petechiae or ecchymoses are not unusual and serious gastrointestinal hemorrhage is not rare Night sweats and slight fever occur in an appreciable number of patients

chemicals pancytopenia and hemorrhagic tendency may be similar to the findings in patients with the aplastic anemia syndrome the presence of the splenomegaly and the character of the cells in the peripheral blood suffice to distinguish between the two conditions

TREATMENT AND COURSE

There is no specific treatment for this disorder which is usually a chronic one that continues over a period of one to twenty years or more [1 1 1 1 1 8 1 7] Weakness recurrent anemia and complaints referable to the enlarged spleen are the most common symptoms but severe gastrointestinal hemorrhages occur occasionally Blood transfusions which constitute the main form of therapy are usually given as often as indicated by the patient s symptoms Neither iron nor vitamin B_{12} is of any value in the *uncomplicated case* Splenic irradiation has been recommended as a form of therapy for patients with discomfort from enlarged spleens accompanied by leukocytosis because of the results seen in seven patients [1 4] Such therapy has also been considered contraindicated because of potential damage to an area of extramedullary hematopoiesis [117] The effectiveness of this type of therapy could not be evaluated in one group of 14 patients [1 8]

For many years splenectomy was considered contraindicated because of the high operative *mortality rate and because it appeared that the operation* removed a needed area of hematopoiesis [1 3] A different opinion was expressed by Green Conley Ashburn and Peters [1 0] who reported that in a series of 24 patients splenectomy was rarely harmful and sometimes produced beneficial effects Splenectomy is most likely to be helpful in the patients with hemolytic anemia who require large numbers of transfusions and in those with associated thrombocytopenia [1 6] Since there is appreciable risk associated with the operation in patients with this disorder splenectomy is not indicated in the patients who can be managed adequately by other forms of treatment In elderly patients and those with very high platelet counts splenectomy is contraindicated because of the risk of operation and the chance of postoperative complications such as thrombosis and hemorrhage secondary to thrombosis

Corticosteroids are sometimes very effective in patients with a *marked* hemolytic element and in those with thrombocytopenia and bleeding [116 127 1 8] A favorable response to these drugs has been considered an indication that splenectomy will be beneficial [116] The effectiveness of androgens in 6 patients with agnogenic myeloid metaplasia has been reported recently [131] Increased erythropoiesis which was delayed four to eight weeks was manifested by increased utilization of radioactive iron and an increase in erythrocyte counts and red cell volume that made transfusions unnecessary while the patients *were treated Because of striking results that were noted in* some of the patients this form of treatment merits further investigation and evaluation

this disorder but such a change has not been found in patients with leukemia who have been treated similarly[1 7] Eosinophilia monocytosis and basophilia sometimes occur in myelofibrosis[1 3 1 8]

The platelets are often large and the total count varies widely in different patients In the series of 56 patients the count was decreased in 13 patients normal in 13 and increased in 30[1 8] Neither a marked reduction to less than 20 000 per cu mm nor a marked increase to over a million per cu mm is unusual

Specimens of bone marrow obtained by aspiration are usually very hypocellular[116 1 7 1 8] but occasionally the marrow appears normal or hypercellular Bone marrow obtained by surgical biopsy and marrow specimens seen at autopsy usually are hypocellular and show increased fibrosis[116 128 13] However some marrows appear normal and others are hyperplastic or contain areas of hyperplasia[1 1 1 6 13 136] Splenic aspiration[116 1 8] and hepatic puncture usually reveal extramedullary hematopoiesis in the form of nucleated red cells young granulocytes and megakaryocytes

Roentgenograms of the bones particularly of the long bones may show a decreased density of the inner surface of the central area of bone and patchy irregular densities in the spongiosa[13] Increased bone density was demonstrated on roentgenograms in one fourth of the patients in one series[128]

DIFFERENTIAL DIAGNOSIS

When a patient presents with splenomegaly and anemia associated with nucleated red cells and immature granulocytes in the peripheral blood the important differential diagnosis is between chronic myelocytic leukemia and the myeloid metaplasia syndrome The appearance of marked poikilocytosis in the smear is suggestive of myeloid metaplasia Failure to obtain a specimen that is consistent with leukemia by marrow aspiration strongly suggests that leukemia is not present however the diagnosis of leukemia cannot be discarded in this circumstance because hypocellular samples and dry taps are sometimes obtained in patients with leukemia even when the marrow is packed with cells The distinction between the leukemia and myeloid metaplasia sometimes cannot be made until after a surgical biopsy of the bone marrow or a needle aspiration of the spleen or liver Sometimes even after these studies the evidence is inconclusive and the distinction between these two disorders cannot be made with assurance until after a longer period of observation

If leukemia appears to be excluded further study for possible causes of the secondary type of extramedullary hematopoiesis such as carcinomatosis tuberculosis and other disorders is indicated If these appear to be excluded and the appearance of the bone marrow biopsy is consistent a diagnosis of myelofibrosis or agnogenic myeloid metaplasia is made A history of previous exposure to toxic substances or of preceding polycythemia very favors this diagnosis Although the age of the patient the history of exposure to

chemicals pancytopenia and hemorrhagic tendency may be similar to the findings in patients with the aplastic anemia syndrome the presence of the splenomegaly and the character of the cells in the peripheral blood suffice to distinguish between the two conditions

TREATMENT AND COURSE

There is no specific treatment for this disorder which is usually a chronic one that continues over a period of one to twenty years or more[113, 114, 116, 117] Weakness recurrent anemia and complaints referable to the enlarged spleen are the most common symptoms but severe gastrointestinal hemorrhages occur occasionally Blood transfusions which constitute the main form of therapy are usually given as often as indicated by the patients symptoms Neither iron nor vitamin B_{12} is of any value in the uncomplicated case Splenic irradiation has been recommended as a form of therapy for patients with discomfort from enlarged spleens accompanied by leukocytosis because of the results seen in seven patients[114] Such therapy has also been considered contraindicated because of potential damage to an area of extramedullary hematopoiesis[117] The effectiveness of this type of therapy could not be evaluated in one group of 14 patients[118]

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ILLUSTRATIVE CASE

A 40 year old white librarian was largely asymptomatic when an "abdominal tumor" was found on routine physical examination in October 1945 At that time the erythrocyte count was 6 6 mil per cu mm and the hematocrit 62 per cent A diagnosis of polycythemia vera was made and during the next four years she received a total of 3 250 r of x radiation to the spleen and a total of 460 r over the long bones Phlebotomies were performed at frequent intervals Following treatment with radiophosphorus in 1950 she had a good symptomatic remission and in 1953 the blood values were hct 49 per cent hb 12 7 gm per 100 ml rbc 6 mil per cu mm wbc 13 000 per cu mm platelets 516 000 per cu mm During the late spring and summer of 1954 the patient noticed progressive weakness easy fatigue slight dyspnea on exertion easy bruisability and recurrent left upper quadrant pain Because of these symptoms she was admitted to the hospital on August 2 1954

Physical Examination Loud systolic murmurs were heard in the aortic area and in the mitral region The liver was palpable three fingerbreadths below the right costal margin the spleen filled the entire left side of the abdomen The remainder of the physical examination was non contributory

Laboratory Examination Admission blood studies disclosed hct 28 per cent rbc 2 8 mil per cu mm hb 8 4 gm per 100 ml retics 0 7 per cent wbc 7400 per cu mm differential count (per cent) myelocytes 2 juveniles 2 bands 7 segs 68 lymphs 18 monocytes 1 eosinophils 1 basophils 1 There were 5 nucleated red cells per 100 white cells The serum bilirubin was 0 54 mg per 100 ml Bone marrow obtained by aspiration and also by surgical biopsy of the iliac crest revealed marked hypoplasia

Treatment and Course The patient responded moderately well to steroid therapy but in the fall of 1955 her symptoms and anemia increased A hemolytic factor was demonstrated by finding an average daily fecal urobilinogen excretion of 245 mg per day over a four day period at a time when the hemoglobin was 8 8 gm per 100 ml Eight months later the anemia was more severe and the fecal urobilinogen excretion was measured at 380 mg per day Because of the hemolytic element splenectomy was performed in September 1956 The spleen weighed 1975 grams Microscopic study of the spleen revealed the presence of many islands of myeloid tissue associated with evidence of erythropoiesis and an increased number of megakaryocytes The findings were considered to be characteristic of myeloid metaplasia The patient did well symptomatically following splenectomy and six months later in April 1957 hemoglobin was 9 4 gm per 100 ml red count 3 mil per cu mm hematocrit 31 per cent platelets 500 000 per cu mm and reticulocyte count 5 1 per cent Immature cells of the myelocytic series and eosinophilia were present in the peripheral blood Daily fecal urobilinogen excretion (average of a 4 day period) was found to be 125 mg

In the fall of 1957 the patient s symptoms changed and from then on heart failure was a problem In addition to the systolic murmurs at the base and apex which had been noted throughout her course a low pitched rumbling

presystolic murmur was heard in the mitral area She became increasingly refractory to treatment and in April 1958 she died after an episode of abdominal pain

At autopsy the usual manifestations of congestive heart failure were present In addition there was marked coronary arteriosclerosis as well as calcific aortic valvulitis and calcification of the annulus fibrosus of the mitral valve Of particular interest was the presence of generalized fibrosis of the bone marrow associated with areas of hypercellularity and evidence of extramedullary hematopoiesis in the liver

Comment This patient s course is similar to that seen in many patients with myelofibrosis At one time she had well developed polycythemia vera which was treated with phlebotomies x radiation and radiophosphorus About eight years after the polycythemia was first recognized she developed anemia This was associated with fibrosis in the bone marrow marked splenomegaly the presence of immature leukocytes and red cells in the peripheral blood and evidence of hematopoiesis in the spleen Splenectomy produced only moderate temporary benefit and the patient succumbed to recurrence of the anemia and heart failure several years later The relationship of the myelofibrosis to the preceding polycythemia vera and to the irradiation therapy is interesting but difficult to interpret The occurrence of both syndromes in the same patient has led some to consider that both these disorders are examples of myeloproliferative syndromes

ANEMIA ASSOCIATED WITH ACUTE BLOOD LOSS

Anemia that is due to the sudden loss of a large volume of blood may occur in a variety of circumstances Among these are trauma postoperative hemorrhage ruptured ectopic pregnancy ruptured aneurysm ruptured esophageal varices and ulcerative lesions of the gastrointestinal tract Massive bleeding occurs spontaneously or after minor trauma in patients with the various hemorrhagic disorders The clinical picture that the patient presents depends on the amount location and acuteness of the bleeding as well as the nature of the underlying disorder and the previous condition of the patient The usual symptoms of an acute hemorrhage are sudden weakness dizziness pallor and sweating The marked variability in the manifestations is shown by the fact that in some patients syncope is the initial symptom while in others the passage of bloody or tarry stools may be the only indication that a hemorrhage has occurred

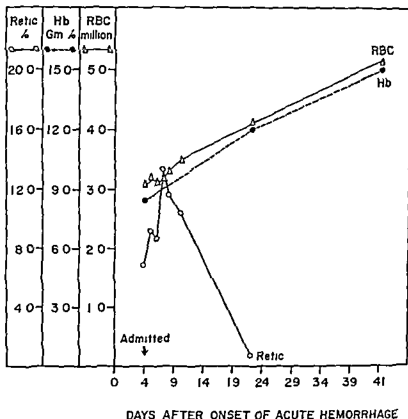


Figure 26 Chart showing hematologic response to acute hemorrhage The peak reticulocyte response of 13 per cent occurred on the seventh day The hemoglobin level and erythrocyte counts returned to normal during the fifth week

The changes in the peripheral blood depend on the time that has elapsed since the hemorrhage the size and location of the hemorrhage and the nature of the underlying disease The amount of blood that is lost can be measured better by a determination of the blood volume than by the hematocrit reading because the latter may not reach its lowest point until 48 or 72 hours after the hemorrhage [137] Unless preceding iron deficiency or a brisk reticulocytosis is present the anemia is usually of the normocytic type Polymorphonuclear leukocytosis accompanied by an increase in the younger forms occurs after a few hours Reticulocytosis begins in two or three days and reaches a peak in from four to seven days [138] Reticulocyte counts as high as 12 or 14 per cent often follow an acute hemorrhage severe enough to reduce the red blood count to about 3 0 mil per cu mm (Fig 26) Hemorrhage into the tissues or body cavities may be followed by rises in both reticulocytes and serum bilirubin If bleeding has ceased an increase in the erythrocyte count becomes evident by the time the reticulocytosis reaches its maximum the count continues to rise even without treatment, until the

normal level is reached usually in from four to six weeks The erythrocyte count is reported to return to a level of 4 5 mil cu mm within about 33 days after an acute episode irrespective of the size of the hemorrhage [139]

There are two important aspects in the diagnosis of the patient with an acute hemorrhage These are the recognition of acute blood loss as the cause of anemia and the determination of the cause of the bleeding Recognition of the hemorrhage usually presents no difficulties but at times its severity may not be appreciated unless it is remembered that the hematocrit reading may give little or no indication of the amount of blood that has been lost If the stool has not been examined for occult blood an elevated reticulocyte count associated with a normocytic anemia or an elevation of the blood urea level may be the first clue to a recent hemorrhage In order to determine the cause of the hemorrhage roentgenographic study of the gastrointestinal tract liver function tests special studies for blood dyscrasias and even surgical exploration may be necessary

Treatment of acute hemorrhage is often the treatment of shock by means of intravenous fluids vasopressor agents and blood transfusions Other *treatment is directed toward the underlying disease Unless the patient's iron stores have become depleted because of previous bleeding or some other factor the hemoglobin and red cell count will return to normal levels after* a moderate sized hemorrhage without therapy with iron

ILLUSTRATIVE CASE

This 41 year old carpenter enjoyed good health until eight months prior to admission when he developed symptoms of a duodenal ulcer which re sponded to diet and medication After he had been asymptomatic for six months the symptoms of ulcer recurred before his admission to the hospital Four days prior to admission the patient noticed marked weakness and sweating followed by the passage of tarry stools Two days prior to admis sion he noticed palpitation and more pronounced weakness Following this he consulted his physician who referred him to the hospital with a diagnosis of gastrointestinal hemorrhage

Physical Examination Blood pressure 128/80 pulse 80 weight 207 height 74 inches temperature 99 There was moderate tenderness on pressure in the epigastric region The remainder of the physical examination was normal

Laboratory Examination Initial blood studies showed hb 8 3 gm per 100 ml hct 24 per cent rbc 3 1 mil per cu mm retics 6 7 per cent wbc 21 000 per cu mm platelets 760 000 per cu mm Differential count (per cent) bands 1 segs 71 lymphocytes 23 monocytes 5 there were 2 nucleated red cells per 100 white cells A stool specimen was almost black in color and gave a strongly positive benzidine reaction A barium study of the gastro intestinal tract was performed without palpation and revealed considerable duodenal scarring with other changes that suggested active ulceration

A diagnosis of duodenal ulcer with hemorrhage was made The patient

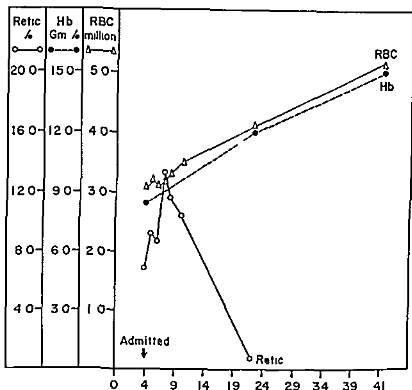


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poral vessels and the dyspnea on dancing and climbing stairs Iron was first used in the treatment of the anemia of chlorosis in the seventeenth century by *Thomas Sydenham* an English clinician In 1889 Hayem recognized that the anemia of iron deficiency is characterized by erythrocytes that are smaller than normal and contain a smaller than normal amount of hemoglobin [1, 9] Most of the progress in understanding iron metabolism and the anemia of iron deficiency has been made in the past two decades since Widdowson and McCance showed that the body normally does not excrete *significant amounts of iron* [1, 6] *Many of the advances have been* possible only because radioactive iron has been available as an investigative tool

MECHANISM OF DISEASE

Iron metabolism which must be understood if the patient with iron deficiency anemia is to be managed properly is discussed in Chapter II

Iron deficiency occurs in a number of clinical conditions and various mechanisms may be involved An improper diet is rarely the sole cause of iron deficiency except in infancy However an inadequate intake may be an important contributing factor in certain situations particularly during *the growth of children and in pregnancy when there is an increased need* for iron The commonest cause of iron deficiency anemia in both men and women is chronic blood loss In adult males hypochromic microcytic anemia *is nearly always the result of chronic blood loss from the gastrointestinal* tract In women gastrointestinal bleeding excessive vaginal bleeding and pregnancy are the commonest causes of iron depletion Patients who have undergone partial gastrectomy form a special category that may develop iron deficiency for it has been demonstrated that in such patients the absorption of dietary iron is markedly reduced and over a period of years the iron stores may become depleted [14, 13] The state of iron stores is an important factor in the development of anemia The loss of small quantities of blood such as occurs in normal menstruation may produce severe anemia in a patient whose iron stores are low yet much more severe bleeding may produce only transient anemia in another patient whose reserves of iron are normal

Abnormalities other than anemia occur at times in patients with iron deficiency Most common among these are fissuring about the mouth trophic changes in the fingernails and dysfunction of the esophagus These abnormalities have been reported as manifestations of iron deficiency in the absence of anemia because these lesions have been found in non anemic patients and improvement in the lesions has followed promptly after the administration of iron salts [1, 3] A similar response to treatment with iron has been reported in non anemic patients who had normal levels of serum iron Even the correction of achlorhydria which is present in about 50 per cent of patients with iron deficiency anemia, has been reported after iron therapy [148] A different result was found in a more recent study of a group of patients with iron deficiency anemia [1] No improvement in the histologic appear

was treated with ulcer diet sedatives alkali and antispasmodics No occult blood was demonstrated in the feces after the third day in the hospital *Transfusions* and iron were not administered When the patient was discharged on the eighth day the hematocrit was 28 per cent red count 3 6 mil per cu mm leukocytes 14 000 per cu mm reticulocytes 8 3 per cent The response of the peripheral blood to the acute hemorrhage while in the hospital and after discharge is shown in Figure 26 Six weeks after the hemorrhage the hematocrit hemoglobin and erythrocyte count were normal

Comment This middle-aged man who had previously had symptoms of a duodenal ulcer was admitted four days after the onset of an acute hemorrhage At this time increases in reticulocytes leukocytes and platelets were already evident The patient made a good spontaneous recovery without *transfusions or other* antianemic therapy The reticulocytes reached a peak of 13 per cent (425 000 per cu mm) on the third day after admission the seventh day after the onset of his acute hemorrhage A rise in erythrocyte count was evident on the fifth day after admission (ninth day after the hemorrhage) and continued in an uninterrupted fashion until the count reached normal between five and six weeks after the initial hemorrhage The level of 4 5 million red cells was reached by the thirty second day after the hemorrhage

ANEMIA OF IRON DEFICIENCY

Anemia that is due to iron deficiency has been described under various names Many of the terms that at present appear to be no more than different names for iron deficiency in special circumstances were used to indicate the various syndromes before the nature of the mechanism responsible for the anemia was appreciated The following terms have been used secondary anemia idiopathic hypochromic anemia chlorosis chlorotic anemia hypochromic anemia of pregnancy hypochromic anemia of prematurity and *adolescence iron deficiency anemia*

An interesting account of the history of iron in medicine has been published by Fowler[14] According to this account the presence of iron in the blood was first discovered by Lemery and Geoffrey in 1713 This discovery did not come until sometime after iron deficiency anemia had been described and iron had been used as a therapeutic agent Iron deficiency anemia was first described by Johannes Lange of Basle in 1554 as "De Morbo Virgineo"[1 1] *In this description of the syndrome that was later called* "chlorosis" Lange noted that the disease was peculiar to virgins and described the change from rosiness of the cheeks to pallor the pulsation of the tem

poral vessels and the dyspnea on dancing and climbing stairs Iron was first used in the treatment of the anemia of chlorosis in the seventeenth century by Thomas Sydenham an English clinician In 1889 Hayem recognized that the anemia of iron deficiency is characterized by erythrocytes that are smaller than normal and contain a smaller than normal amount of hemoglobin[10] Most of the progress in understanding iron metabolism and the anemia of iron deficiency has been made in the past two decades since Widdowson and McCance showed that the body normally does not excrete significant amounts of iron[16] Many of the advances have been possible only because radioactive iron has been available as an investigative tool

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ance of the gastric mucosa or in the acid secretion occurred after a year of treatment with iron. Further evidence of a discrepancy between the iron content of the blood and the iron content of the tissues, particularly iron containing enzymes, is afforded by the work of Beutler who found that in rats that were made iron deficient by bleeding and special diets the decrease in the cytochrome C of the liver and kidney greatly exceeded the decrease in hemoglobin.[111] Other studies have shown that not all iron containing enzymes are so dependent on adequate supplies of iron. The concentration of catalase has been found to be normal in human red cells even under conditions of severe iron need.[110]

CLINICAL MANIFESTATIONS

The symptoms that occur in the patient with iron deficiency anemia may be related to the primary disease or to anemia. Some patients consult a physician because they have noticed abnormal blood loss such as menorrhagia or rectal bleeding. Other patients who have not noted any abnormal blood loss visit a physician because of symptoms that are related to the anemia per se, such as weakness, easy fatigability, dyspnea on exertion and palpitation. In addition to the usual symptoms of anemia, patients with iron deficiency sometimes complain of dysphagia, sore tongue, sore mouth and brittle nails. Still other patients seek help because of symptoms that are caused by a lesion of the gastrointestinal tract, such as a duodenal ulcer or carcinoma of the bowel, and the presence of anemia is not suspected until a blood count is done.

Physical examination in some patients reveals no abnormality and in others simply pallor of the skin and mucous membranes. At times fissuring at the angles of the mouth, atrophy of the papillae of the tongue, and glossitis are evident. In severe longstanding iron deficiency the nails may be "spoonshaped" and abnormally brittle. The spleen is often palpable in children and adults with iron deficiency but does not extend more than a few centimeters below the left costal margin in the uncomplicated case. Throbbing headaches sometimes occur when the hemoglobin is greatly reduced and disappear when the anemia is corrected. When the anemia is moderately severe an apical or precordial systolic murmur is usually present. Other cardiovascular abnormalities, such as cardiac dilatation and congestive failure, occur most often in younger children and in older persons with some coexisting heart disease.

LABORATORY EXAMINATION

The anemia of severe iron deficiency is characteristically hypochromic and microcytic (MCV is less than 80 cu. microns, MCH less than 30 micromicrograms and MCHC less than 30 per cent). Much lower values are often found. In the majority of patients with iron deficiency, study of a stained smear of the peripheral blood reveals that most of the erythrocytes are

smaller than normal and show an increase in the central pallor that may be extreme (hypochromic microcytes) other erythrocytes appear normal in color and some show polychromatophilia if erythropoiesis is active Poikilocytosis is often evident and elongated cigar forms" are seen frequently Occasionally nucleated red cells are present The leukocyte count, platelet count and reticulocyte counts are usually normal Erythropoiesis is normoblastic in type but the cellularity of the marrow may appear normal reduced or increased

In patients with less severe iron deficiency the anemia is not always hypochromic and microcytic it may be hypochromic and normocytic or normochromic and normocytic In such patients other laboratory procedures may be helpful in establishing a diagnosis of iron deficiency The presence of iron deficiency usually is accompanied by a reduction in the level of the serum iron generally to less than 40 μg per 100 ml (normal 50 150) and an increase in the latent iron binding capacity of the serum to two or three times the normal value (normal 200 300 μg per cent) Study of bone marrow that is obtained by aspiration and stained for iron may also provide a reliable index of the state of the iron stores particularly if the stores are low [14] When anemia is due to iron deficiency the serum bilirubin is not elevated and the 24 hour fecal urobilinogen excretion is usually lower than normal because the mass of the circulating hemoglobin is reduced Achlorhydria occurs commonly in patients with this disorder The free erythrocyte protoporphyrin is increased in iron deficiency [144]

DIFFERENTIAL DIAGNOSIS

The first step in making a diagnosis of this type of anemia is to establish the presence of iron deficiency and the next is to determine the cause If the anemia is hypochromic and microcytic the commonly seen iron deficiency must be distinguished from the uncommon thalassemia and the rare pyridoxine deficiency Thalassemia is characterized by a family history of anemia the occurrence of many target cells on the stained smear and an elevated reticulocyte count the spleen is usually enlarged and may extend below the level of the umbilicus Determination of the serum iron level and examination of properly stained marrow preparations fail to reveal any iron deficiency Fetal hemoglobin is usually found Pyridoxine deficiency which is rare in man is characterized by a hypochromic microcytic anemia but even though the erythrocytes appear hypochromic the level of the serum iron is elevated and administration of iron produces no improvement in the anemia [149] In the milder degrees of iron deficiency the anemia may be hypochromic normochromic normocytic or slightly microcytic rather than hypochromic microcytic This is most likely to occur when severe deficiency has not developed or when the severe deficiency has been only partly corrected In such circumstances the demonstration of a low serum iron level and/or an increase in the latent iron binding capacity or a histologic demonstration

of low iron stores in the tissue often establishes the presence of iron deficiency

If the presence of iron deficiency has been established an effort is made to find the cause of the deficiency This aspect of the diagnosis is discussed in Chapter II Faulty diet may be an acceptable explanation in infants and very young children but in adults a search must be made for the cause of the blood loss The absence of occult blood from a single specimen of feces does not exclude chronic or recurrent gastrointestinal bleeding from consideration Unless the cause of the iron deficiency is clear cut most patients particularly those who are middle aged or older will need a roentgenographic study of the upper and lower gastrointestinal tract Almost any ulcerative lesion in the esophagus stomach small intestine or large bowel can be responsible for chronic bleeding and iron deficiency If a lesion of the gastrointestinal tract is suspected but not demonstrated at the time of the initial examination the appropriate studies are often repeated because of the importance of demonstrating any lesion particularly a neoplasm that might require surgical correction In women particularly those of child bearing age the decision must be made whether or not the history of vaginal bleeding and pregnancies gives an adequate explanation of the anemia Roentgenographic investigation of the gastrointestinal tract is often indicated but the risk of irradiation to the ovaries or to an unsuspected early pregnancy should not be ignored

TREATMENT COURSE AND PROGNOSIS

The most important aspect of the treatment of iron deficiency anemia is the correction of the cause This is much more important than the correction of the anemia unless the patient is critically ill The prognosis is related more to the underlying disease than to the severity of the anemia which may have little or no influence on the outcome

In patients with anemia that is due to iron deficiency the administration of ferrous salts is followed by a reticulocytosis during the first week (Fig 27) The magnitude of reticulocyte response which is usually in the range of 4 to 10 per cent depends on the initial hemoglobin level and not on the level of the erythrocyte count Unless complicating factors are present the reticulocytosis is followed by the return of the hemoglobin and red cells to normal levels within a period of six to ten weeks If the therapy with iron is successful the hemoglobin level should increase 2 0 gm per 100 ml in the first three weeks of treatment [146, 154] The levels of iron and latent iron binding capacity which probably reflect the state of the stores of iron, return to normal much more slowly Treatment probably should be continued until this occurs but such complete therapy may require small amounts of iron salts orally for a long period particularly if the patient is either a premenopausal woman or has had a partial gastrectomy Prolonged treatment of this type is considered impractical by some [153]

The anemia of iron deficiency is usually corrected by the oral administra

tion of 0 2 or 0 3 gm of ferrous sulfate or ferrous gluconate three times a day If a small amount of the drug is given initially and the dosage is increased gradually gastrointestinal upsets rarely occur Some patients tolerate one preparation better than another and if one preparation is causing undesirable griping diarrhea or other symptoms another compound should be substituted. Iron is less likely to cause gastrointestinal symptoms if it is given after meals Although this is not an optimal time for iron absorption adequate absorption usually occurs if the medication is given at this time A single tablet of ferrous gluconate or ferrous sulfate at bedtime generally produces a satisfactory response and many patients find it simpler and more convenient to follow this schedule

Although iron salts administered orally correct the anemia in most patients the method of treatment is not an efficient means of correcting the iron stores because iron absorption decreases when the blood returns to normal In order to replenish the iron stores by the oral route it is often necessary to continue treatment for months or years For this reason parenteral preparations are sometimes employed Saccharated iron oxide given intravenously in an initial dose of 20 to 50 mg and in subsequent daily doses of 100 to 200 mg produces excellent therapeutic results[143] More recently the intramuscular therapy with iron dextran which has been found to be

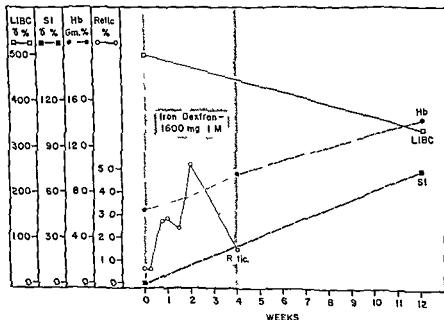


Figure 27 Chart showing hematologic response of a patient with iron deficiency anemia to treatment with iron The reticulocyte peak which is not as great as that seen after specific treatment in pernicious anemia, occurs before there is an increase in the levels of hemoglobin and serum iron and a decrease in the latent iron binding capacity

largely free from local reactions has gained in favor[1,4] Injections of 100 to 500 mg may be given weekly or several times a week The total amount given in a course of intramuscular treatment is usually between 1500 and 5000 mg an amount sufficient to correct the anemia and replenish the iron stores

ILLUSTRATIVE CASES

Case 1

A 74 year old white housewife was referred in April 1956 for evaluation of anemia and weight loss of 20 pounds during the preceding year About one and a half years prior to admission she had an episode of constipation cramping midabdominal pain and vomiting Roentgenographic examinations of the gallbladder and upper gastrointestinal tract were negative Her attacks recurred with increasing frequency and about six weeks before admission were occurring once or twice a week At this time the patient was found to be anemic and was treated with iron

Physical Examination The skin and mucous membranes were pale The right side of the abdomen was moderately tender and a firm movable 6 by 10 cm mass was felt in the right lower quadrant The liver edge was palpable 2 cm below the right costal margin

Laboratory Studies Admission blood studies disclosed the following hct 26 per cent hb 6 7 gm per 100 ml rbc 3 4 mil per cu mm wbc 4900 per cu mm serum iron 11 5 μg per 100 ml unsaturated iron binding protein 455 μg per 100 ml The feces gave a strongly positive benzidine reaction

Barium enema examination revealed a 5 or 6 cm constricted area in the ascending colon and another filling defect in the sigmoid colon After blood transfusions operation was performed A carcinoma of the ascending colon and a carcinomatous polyp in the descending colon were found Both lesions were resected and convalescence was uneventful

Three months later the hct was 43 per cent hb 12 6 gm per 100 ml rbc 4 4 mil per cu mm plasma iron 99 μg per 100 ml and unsaturated iron binding protein 270 μg per 100 ml

Comment At the time of admission this patient had evidence of severe iron deficiency in the form of hypochromic microcytic anemia associated with a decrease in the level of serum iron and an increase in the unsaturated iron binding capacity The most important aspect of the diagnosis in a patient with anemia of this type is the demonstration of the cause of the anemia Usually as was the case in this patient the iron deficiency is the result of chronic blood loss In adult males and elderly women chronic unrecognized blood loss is nearly always the result of a lesion in the gastrointestinal tract This patient had two primary malignant lesions in the colon It appears likely that the lesion in the ascending colon was responsible for the blood loss while the tumor in the sigmoid colon was responsible for the symptoms of partial intestinal obstruction In all probability a more thorough examina

tion at the time her symptoms began a year and a half before admission would have revealed a lesion in the colon Fortunately this patient appeared to have no metastases when her bowel resections were performed despite a delay in diagnosis that might well have been fatal

Case 2

This 69 year old white housewife was admitted with the chief complaint of "anemia of two years duration She related that for two years she had felt weak nervous and slightly dizzy She had been treated irregularly for two years with liver extract and iron Despite this therapy some degree of anemia persisted and she was referred for further study Careful questioning elicited no history of weight loss gastrointestinal symptoms or abnormal bleeding

Physical Examination The patient was well developed well nourished and somewhat obese General examination revealed no specific abnormalities but the pelvic examination revealed an erosion of the cervix

Laboratory Examination Admission blood studies hb 9 1 gm per 100 ml rbc 3 8 mil per cu mm hct 31 per cent retics 1 9 per cent The leukocyte count platelet count and differential count were normal Bone marrow examination disclosed a hypercellular marrow with a normoblastic type of erythropoiesis Repeated stool examinations were consistently positive for occult blood

Gastrointestinal x ray study revealed a hiatus hernia that was about 10 cm in the greatest diameter Barium enema examination revealed a lesion at the hepatic flexure which was associated with some narrowing over an area of 2 or 3 cm Proctoscopic examination disclosed no lesion Biopsy of the cervix was reported as showing an epidermoid carcinoma

Treatment and Course The patient was transfused with three units of blood and was operated upon nine days after admission A right colectomy was performed and the patient stood the procedure well At the time of operation there was no evidence of metastases from the lesions in bowel or cervix Histologic study of the bowel tumor revealed mucinous adenocarcinoma grade III extending through the thickness of the wall The regional lymph nodes were free of tumor The patient's convalescence from the laparotomy was uneventful and sixteen days after her right colectomy radium was applied to the cervix She received 6000 mg hours using six sources and the following month she received 6000 r of external radiation

Comment This patient illustrates many instructive points She was treated for two years for anemia with iron and liver extract without any effort being made to discover the cause of the anemia This treatment altered the morphologic characteristics of the red blood cells without correcting the anemia This case illustrates the importance of determining the cause of the anemia before treatment When the gastrointestinal tract was investigated two possible bleeding sites were found The hiatus hernia was found first but this lesion occurs so frequently in this age group that it was thought

important to study the lower gastrointestinal tract. This was done and the carcinoma of the colon was discovered. Fortunately the lesion was operable despite the duration of the illness. The finding of a carcinoma of the cervix on the routine examination emphasizes the importance of a complete examination in all patients.

HEREDITARY SPHEROCYTOSIS

Synonyms: Congenital Hemolytic Icterus, Acholuric Jaundice, Congenital Hemolytic Anemia, Familial Spherocytosis, Spherocytic Anemia

HISTORY

The history of this disorder has been reviewed recently by Dreyfus.[168] Formerly credit for the first detailed clinical description of "congenital hemolytic icterus" was usually given to Minkowski's report in 1900.[1 3] Because of Chauffard's discovery of two of the characteristic features of the disease, namely reticulocytosis and the increased hypotonic saline fragility of the red cells,[164, 16, 1 3] the disorder was sometimes referred to as the hemolytic anemia of the Chauffard and Minkowski type. Vanlair and Masius' report in 1871, in which spherocytes were described, has been rediscovered only recently[164] and, as a result, credit for the first clear description of this disease has been given to these authors.[164, 1 0, 16]

MECHANISM OF DISEASE

Hereditary spherocytosis is believed to be inherited as a Mendelian dominant.[16, 180] According to this concept at least one parent and one half the offspring of the patient should be affected. However, apparent exceptions have been observed in families that were studied carefully.[1, 1 6] Consideration of these apparent exceptions has led to the suggestion that they may be explained in one of the following ways: (1) the testing method may be inadequate to detect the anomaly in the parents and siblings possibly because of factors of gene penetrance or expressivity; (2) some patients may develop the spherocytosis as a result of gene mutation; (3) the disease in the exceptional cases that appears to be hereditary spherocytosis is in reality similar to it but is not identical with it; (4) the legal parents are not the biological parents.[16, 186] The first and third possibilities are considered most likely.[1 7, 15, 186]

Patients with hereditary spherocytosis manifest the evidences of a hemolytic process such as increased fecal urobilinogen excretion elevation of the indirect reacting serum bilirubin in the plasma splenomegaly reticulocytosis and normoblastic hyperplasia in the bone marrow Although the anemia is cured by splenectomy an intracorpuscular defect has been shown by Dacie and Mollison[165] to be the primary factor in the increased hemolysis They demonstrated that erythrocytes from normal donors survived from 100 to 130 days when transfused into patients with hereditary spherocytosis in contrast erythrocytes that were obtained from a patient with hereditary spherocytosis four days prior to splenectomy and one year after splenectomy disappeared from the circulation in 14 days and 19 days respectively when transfused into normal recipients

Ever since the increased osmotic fragility of the erythrocytes in hereditary spherocytosis was first described efforts have been made to find some link between this peculiarity and the increased destruction of the erythrocytes in vivo Haden demonstrated that the increased susceptibility of the erythrocytes in the disease to hypotonic saline is related to their increased thickness and "microspherocytic" shape[171] The observation that microspherocytosis and increased osmotic fragility persisted after splenectomy suggested that the defects in the red cell were the result of an inherited abnormality of erythropoiesis[1, 1, 174] Ham and Castle found that sterile incubation caused an increase in the spheroidicity and osmotic fragility of human erythrocytes and postulated that abnormally susceptible erythrocytes would be hemolyzed more easily than normal cells by erythrostasis in the spleen which might be compared to incubation in vitro[1] This concept implies selective sequestration of the abnormal "spherocytes in the spleen because normal erythrocytes survive normally in patients with hereditary spherocytosis[165, 169, 173] Such selective sequestration has been demonstrated in experiments that utilized mixtures of different types of cells that could be identified by differential agglutination Identifiable normal erythrocytes were transfused into patients with hereditary spherocytosis prior to splenectomy when the spleen was studied after its removal it was found that the patient's cells had been selectively retained by the spleen In another experiment spleens that had been removed from patients with hereditary spherocytosis were perfused with mixtures of normal erythrocytes and spherocytes from patients with hereditary spherocytosis[169, 157] again it was found that a disproportionately higher number of spherocytes were retained by the spleen Thus it appears that the increased thickness of the erythrocytes which is an inherited abnormality is responsible not only for their increased osmotic fragility but also for their sequestration in the spleen where they are destroyed at an excessive rate

The inherited defect in the spherocytes of this disease is complex Abnormalities in the glycolytic cycle of the cell have been demonstrated by Prankerd Altman and Young who found a smaller flux of P^{32} into ATP and 2 3 DPG associated with an increase in the flux into orthophosphate[178] These authors postulated that the susceptibility of such cells to hemolysis

may be a consequence of a deficiency of high energy phosphate bonds An increased rate of the transport of sodium across the cell membrane has also been demonstrated in the erythrocytes in this disease [1 9] Whether or not the abnormal rates of sodium transport are due to the defective glycolysis of the cells is uncertain The fact that quantitative differences were found in different patients with hereditary spherocytosis as well as in asymptomatic kindred has led to the suspicion that patients with hereditary spherocytosis may not form a homogeneous group but may vary in the number of genetically determined biochemical abnormalities that are present [1 9]

CLINICAL MANIFESTATIONS

Hereditary spherocytosis occurs in both sexes, but it is rare in Negroes [160] Clinical evidence of the disease may appear soon after birth or not until after middle age [180, 186]

The severity of the disease varies greatly in different patients In some the clinical manifestations are so mild that the disease is asymptomatic and the patients lead an active normal life In such individuals the diagnosis can be established only after a careful study of the blood because the anemia may be mild or even absent if the bone marrow is able to compensate for the hyperhemolysis More often the patient has the symptoms of a mild chronic illness Some patients do not realize that they have been feeling subnormal until after splenectomy produces an increased sense of well being

In many patients the course of the disease is associated with episodes of severe illness the so called "crisis" These attacks are characterized by the sudden onset of fever abdominal discomfort nausea vomiting and rapidly increasing weakness and pallor Tachycardia and low blood pressure are usually present and shock may develop Some patients appear severely ill and lethargic and lapse into unconsciousness [166] It was formerly thought that the episode of crisis was caused by a marked increase in the severity of hemolysis but the recent studies of such episodes indicate that at least the majority are the result of a combination of decreased erythropoiesis and a continuation of the usual degree of hemolysis [1 8, 166, 1 5] Owren described the occurrence of an "aplastic crisis characterized by rapidly increasing anemia leukopenia and thrombocytopenia that accompanied a fall in the reticulocyte count and a decrease in the plasma bilirubin and the development of an aplastic picture in the bone marrow [17] The occurrence of episodes of this type in several members of a family at the same time suggests that some intercurrent infection is the causative factor [1 8, 168, 1] Other attacks labeled as crisis that are associated with increasing jaundice may be in reality attacks of biliary colic and infection In these circumstances the features of obstructive jaundice and infection are superimposed on those of hyperhemolysis Cholelithiasis occurs in over half the patients over the age of 10 and in only about 5 per cent of those under 10 years of age [1 7] In one

group of 14 patients over 11 years of age cholelithiasis occurred in 12.[156]

The most constant abnormality found on physical examination is splenomegaly of mild or moderate degree. In most patients the spleen is firm and does not extend below the level of the umbilicus but in some patients it fills the entire left half of the abdominal cavity.[167, 184] The liver is often palpable but is rarely significantly enlarged. The patient with hereditary spherocytosis has been described as "*more jaundiced than sick*," but the degree of clinical jaundice is usually mild unless there is associated dysfunction of the biliary tract. Both the pallor and the jaundice may be so mild as to be overlooked on the physical examination. Chronic leg ulcers in the region of the malleoli are reported to occur in 6 per cent of patients[1, 9] but are also said to be rare.[1, 9, 184, 186] The ulcers are usually several centimeters in diameter and are surrounded by an area of hyperpigmentation. In patients with hereditary spherocytosis growth and development are usually normal. Abnormalities such as tower skull, polydactyly, skeletal defects, and infantilism have been reported in this disease but appear to be uncommon.[15]

LABORATORY EXAMINATION

The anemia is usually moderate and erythrocyte counts of 3.0 to 3.5 mil per cu mm are common. The erythrocyte count may be normal if the hemolytic process is fully compensated, or markedly reduced if there is an episode of crisis. In one series the erythrocyte counts ranged from 1.5 to 5.0 mil per cu mm.[15] The MCV is nearly always normal or slightly reduced and the MCH and MCHC are generally normal because the erythrocytes have an increased thickness as well as a reduced cell diameter. On a stained blood smear many of the erythrocytes appear small and heavily stained because of a marked diminution or absence of the central pallor that is seen in normal cells. Macrocytes, which are probably the younger cells, are also present as a rule. A reticulocytosis of from 10 to 20 per cent or more is found regularly unless erythropoiesis is depressed because of an aplastic crisis or for some other reason. In one series of patients the reticulocyte count ranged from 6 per cent to 78.6 per cent.[1] During an aplastic crisis reticulocytes may disappear from the peripheral blood.[168, 1] In the usual patient the bone marrow undergoes hyperplasia of the normoblastic type. During an aplastic crisis the marrow may be either aplastic or unusually cellular, the appearance depends on whether the specimen is obtained near the onset of crisis or during the recovery phase.[1]

Study of the patient discloses other features common to hemolytic anemias of all types, such as an elevation of the level of serum bilirubin, an increase in the excretion of fecal urobilinogen and urinary urobilinogen, and normal or elevated levels of the serum iron. In one series the serum bilirubin level ranged from 0.4 to 5.7 mg per 100 ml, but in 80 per cent it was below 3.0 mg.[186] Levels higher than 4.0 mg per 100 ml are unusual unless complications are present.[184]

Increased fragility of erythrocytes in hypotonic saline solutions has been recognized as a characteristic feature of hereditary spherocytosis. When normal erythrocytes are tested in saline solutions hemolysis begins in concentrations of about 0.45 per cent and is complete at 0.30 per cent. In most patients with hereditary spherocytosis hemolysis begins at concentrations of from 0.50 per cent to 0.75 per cent and is complete at 0.40 per cent. The test has been modified so that results may be expressed quantitatively.[164, 186] When incubated for 24 hours at 37° C erythrocytes from patients with hereditary spherocytosis show a greater increase in osmotic fragility than do normal cells.[186] In mildly affected cases the abnormal osmotic fragility may not be evident until after incubation. Microspherocytes have not only an increased hypotonic saline fragility but also an increased mechanical fragility.[169, 178] The Coombs antiglobulin test is usually negative even during episodes of crisis.

DIFFERENTIAL DIAGNOSIS

The diagnosis may be difficult if the patient is seen for the first time during an episode of aplastic crisis or during an attack of biliary colic when the hemolytic nature of the underlying disorder may not be suspected. As a rule the presence of mild anemia, splenomegaly and slight icterus suggests the diagnosis. The presence of hyperhemolysis is confirmed by finding reticulocytosis, increased urobilinogen excretion and elevation of the indirect reacting bilirubin in the blood.

Hereditary spherocytosis is generally distinguished from other types of hemolytic anemia by the family history and the hypotonic fragility test. If the results of this test are not clear cut, incubation at 37° C for 24 hours may be helpful.[186] However the presence of microspherocytes and increased osmotic fragility do not establish the disorder as hereditary spherocytosis, for these sometimes occur in other types of hemolytic anemia. Increased osmotic fragility has been found in acute hemolytic anemia, myelosclerosis, leukemia, erythroblastosis fetalis, lymphadenoma, ovarian dermoid cyst, lymphosarcoma, pneumonia and after the administration of hemolytic poisons such as acetylphenylhydrazine, sulfanilamide and arsenic.[164] At times study of the blood of siblings and parents is necessary to establish the diagnosis of hereditary spherocytosis in an individual patient. In such a study the incubation procedure is particularly useful. The Coombs antiglobulin test, hemoglobin electrophoresis and the alkali denaturation test are often helpful in distinguishing hereditary spherocytosis from other hemolytic anemias such as the hemoglobinopathies and auto immune hemolytic disease. Hereditary spherocytosis must be differentiated from the unusual congenital nonspherocytic hemolytic anemia which is not benefited by splenectomy. In the latter condition spherocytes and abnormal hypotonic saline fragility do not occur.

TREATMENT

Splenectomy was first performed on a patient with this disease by Wells in 1887 some years before the disease was recognized[167] It has been established since then that the procedure almost invariably relieves the anemia and jaundice[164 35 186] The serum bilirubin level returns to normal promptly and the erythrocyte count generally becomes normal in from two to four weeks (Fig 28) Although spherocytosis is less marked after splenectomy both the spherocytosis and increased osmotic fragility persist after operation[19 186] Operation appears justified in virtually all patients with this disease except those with the mildest form of the disorder unless there are other contraindications to surgery Splenectomy may be done in infancy if the anemia is severe but postponement to late childhood if possible is recommended by Dacie[164 184] If the patient has cholelithiasis cholecystectomy may be performed at the time of splenectomy but at times it may be more prudent to perform the operations separately

Folic acid iron and vitamin B_{12} are without value in the uncomplicated case of hereditary spherocytosis Transfusions may be necessary during

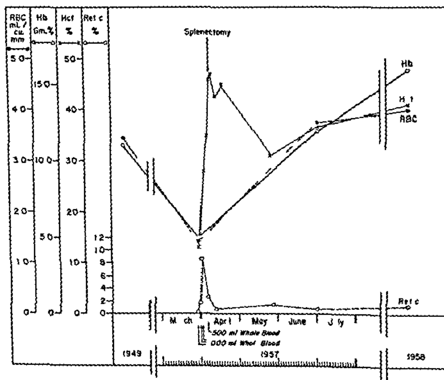


Figure 28 Hereditary spherocytosis showing results of splenectomy The patient was admitted during an aplastic crisis when the reticulocytes were nearly zero despite transfusions the reticulocytes rose within a few days

episodes of crisis particularly if splenectomy must be done at such a time, but do not constitute a regular form of treatment

ILLUSTRATIVE CASE

This patient was first seen at the University Hospital in 1947 at the age of 22 because of acute intoxication with isopropyl alcohol At that time the following abnormalities were noted splenomegaly icterus index 18 units hemoglobin 7 0 gm per 100 ml reticulocyte count 1 7 per cent generalized hyperplasia of the bone marrow He refused further investigation and treatment until 1949 when he returned for more complete studies At that time the liver was palpable 2 cm below the right costal margin and the lower border of the spleen was at the level of the umbilicus

Laboratory Examination Admission blood studies hb 10 gm per 100 ml rbc 3 6 mil per cu mm wbc 6600 per cu mm serum bilirubin *1 5 mg per 100 ml retics 4 9 per cent Numerous microspherocytes were* seen on a smear of the peripheral blood A specimen of bone marrow obtained by aspiration was normoblastic and hyperplastic The daily fecal urobilinogen excretion was 1600 mg Hypotonic saline fragility test beginning hemolysis at 0 48 per cent complete at 0 40 per cent normal control beginning 0 44 per cent complete 0 34 per cent The antiglobulin (Coombs) test was negative

The family history revealed that the patient's father and grandfather suffered from episodes of jaundice The father of the patient age 75 was studied and the characteristic hematologic abnormalities of hereditary spherocytosis were demonstrated

The patient underwent splenectomy and had an uneventful postoperative course He was discharged on the seventh postoperative day when the hematocrit was 39 per cent two months later it was 50 per cent Two years later the serum bilirubin level and hematocrit were normal

Comment This patient demonstrates many characteristic features of hereditary spherocytosis Anemia splenomegaly reticulocytosis bone marrow hyperplasia increased fecal urobilinogen excretion and slight elevation of the serum bilirubin such as this patient had are found in all types of hemolytic anemia The presence of the microspherocytes and the occurrence of a similar disorder in other members of the family indicated that the diagnosis was probably hereditary spherocytosis The diagnosis was confirmed by the hypotonic saline fragility test In many patients with hereditary spherocytosis the results of the test are more abnormal than was the case in this patient but in other patients with the disease incubation is necessary to demonstrate a definite abnormality As illustrated by this patient's subsequent course splenectomy cures the anemia and hyperhemolysis in hereditary spherocytosis Patients with the disease not infrequently need cholecystectomy because the hyperhemolysis if not corrected by splenectomy leads to an increased incidence of cholelithiasis Attacks of biliary colic and obstructive jaundice are not unusual

SICKLE CELL ANEMIA

Synonyms Meniscocytosis, Drepanocytic Anemia, Homozygous Hb S Disease

HISTORY

The first case of sickle cell anemia was described by Herrick in 1910 [08] This patient a 20 year old male Negro from the West Indies had most of the classic clinical and hematologic features of the disease In addition to the sickle cells this patient had cardiac enlargement icterus anemia normoblasts in the peripheral blood and leukocytosis Huck established the fact that the sickling property resides in the cell and not in the plasma [08] and Ponder showed that red cells washed free of hemoglobin do not sickle Hahn and Gillespie demonstrated that sickling is a reversible reaction related to the degree of oxygenation of the hemoglobin in the erythrocyte [03] A most important contribution was made by Pauling Itano Singer and Wells in 1949 when they established that sickle cell anemia is a molecular disease [3] They demonstrated that hemoglobin obtained from patients with sickle cell anemia differed electrophoretically from that present in erythrocytes of normal individuals and concluded that the abnormality resided in the protein portion of the molecule The nature of the defect has been further characterized by Ingram who has shown that the molecule of sickle cell hemoglobin differs from a molecule of normal hemoglobin only by having glutamic acid replaced by valine [10] The studies of Neel and others have shown that the molecular abnormality of hemoglobin is genetically controlled [10] [0] [1]

MECHANISM OF ANEMIA

The sickling property is a gene transmitted abnormality that is heterozygous in persons with the asymptomatic sickle cell trait and homozygous in individuals with sickle cell anemia [10] [0 - 1] This inherited defect is found in the globin portion of the hemoglobin molecule and not in the heme portion [3] Recently it has been shown that sickle cell hemoglobin and normal hemoglobin differ in that the peptide chain in each half of the sickle cell hemoglobin molecule contains valine in a situation where glutamic acid is found in normal hemoglobin [10] Thus it appears that of almost three hundred amino acids present in each half of the hemoglobin molecule hemoglobin S and hemoglobin A differ in only one amino acid This molecu

lar difference the presence of two carboxyl groups in glutamic acid that are absent in valine appears to be adequate to explain the electrophoretic differences in these two hemoglobins In addition efforts have been made to explain the unusual chemical and clinical manifestations of the disease by this molecular peculiarity

The sickle cell hemoglobin tends to crystallize under conditions of lowered oxygen tension [4] and both lowering of the pH and deoxygenation of solutions of sickle cell hemoglobin lead to the formation of crescent shaped tactoids [94] Sickling of erythrocytes which is reversible is thought to be the result of realignment of the hemoglobin molecules in the cell envelope rather than the actual crystallization of the hemoglobin molecules [94] Erythrocytes from patients with sickle cell anemia assume the sickle shape at oxygen tensions that are found in the circulation in vivo but a more marked lowering of the oxygen tension is necessary to produce sickling in individuals with the sickle cell trait It has been assumed that this difference in behavior between the erythrocytes that contain only sickle hemoglobin and those that contain both normal and sickle hemoglobin forms the basis for the different clinical manifestations of sickle cell anemia and the sickle cell trait condition Individuals who have the sickle cell trait are essentially asymptomatic and remain so unless the oxygen saturation of the blood is reduced to an abnormal degree Patients who are homozygous for hemoglobin S have sickle cell anemia a chronic illness characterized by anemia and the occurrence of infarctions in various organs of the body

The anemia which is hemolytic in type is the result of an intracorpuscular defect [158, 193, 194, 9] Erythrocytes from normal individuals survive for about 120 days when transfused into patients with sickle cell anemia while erythrocytes from patients with sickle cell anemia have a much shorter survival time when transfused into normal recipients Studies that utilized the analysis of N^{15} disappearance and fecal stercobilin N^{1} appearance indicate complete random destruction of the red cells a shortened survival time of the erythrocytes (16 days in one patient) and no evidence of more than one population of cells [11] It has been suggested that the excessive destruction of the erythrocytes is the result of the increase in mechanical fragility that has been demonstrated when the erythrocytes assume the sickle cell form [94] According to this explanation the erythrocytes become sickled under the oxygen tensions that occur in the body normally and in this form both the mechanical fragility of the cells and the amount of trauma that they encounter in the circulation are increased The occurrence of transient marrow aplasia is also responsible for a sudden increase in the severity of the anemia in some circumstances [30]

The development of the lesions that occur in the various organs of the body is usually attributed to thrombosis and infarction or to ischemic necrosis and hemorrhage[198, 213] either ischemia or thrombosis would be favored by the packing and stasis of the sickle shaped erythrocytes in the capillaries under conditions of lowered oxygen tension [190] It has been suggested that the assumption of the sickle form is associated with an increase

in viscosity which leads to a "vicious cycle" of increased deoxygenation increased sickling, and increased stasis [9]

CLINICAL MANIFESTATIONS

Sickle cell trait and sickle cell anemia have been found almost entirely in the Negro race The incidence of the sickling trait varies in different parts of the world and is about 9 per cent in American Negroes Sickle cell anemia has a frequency of about one fortieth that of the trait [19]

Sickle cell anemia is characterized by the occurrence of symptoms in virtually any part of the body These symptoms are sometimes bizarre and vary greatly from patient to patient and from time to time in the same patient Most patients with sickle cell anemia both children and adults continue in a mild state of chronic illness with stable levels of erythrocytes and



Figure 9 Typical leg ulcer in sickle cell anemia

serum bilirubin but nearly all patients at one time or another experience episodes of more severe symptoms the attacks of so called crisis At such times the patient may become suddenly ill with severe pain fever and clinical features that suggest a localized lesion in some part of the body Severe disease of the central nervous system or some complication in the skeleton or intra abdominal organs that requires surgical treatment may be suspected More often the onset of these episodes is mild and is characterized by slight fever mild abdominal pain and arthralgia However some patients develop the striking changes in the symptoms that occur during a crisis without any accompanying hematologic changes of significant degree The factors which precipitate these attacks are not known but infection is often a preceding event The anemia and jaundice often increase temporarily during these episodes but return to their previous levels afterward

The most common cutaneous manifestations of the disease are indolent ulcers or scars on the lower legs which can be found on about 75 per cent of the older children or adults with the disease Ulceration is rare in very young children[198] *The ulcers which have sharply defined edges are usually* single but may be multiple and bilateral (Fig 29)

Various types of cardiac abnormalities have been reported in from 50 to 91 per cent of the patients[189, 34] The commonest of these is an apical systolic murmur which occurs in the majority Although rare mitral aortic and pulmonic diastolic murmurs have been reported[34] Cardiac enlargement which often involves both ventricles is a common finding and has been attributed to the chronic anemia[73] Cor pulmonale occurs in some patients possibly as a result of pulmonary infarction and pulmonary hypertension[1, 37] Electrocardiographic abnormalities such as a prolonged PR interval and minor changes in the ST segment and T wave occur but are non specific

Symptoms referable to the genitourinary tract are not common but the complications may be dramatic and serious The most frequent of these is hematuria The cause of the hematuria which occurs in both sickle cell anemia and sickle cell trait even in the absence of infarction is poorly understood The bleeding is usually unilateral recurrent and may be severe Some patients have had operations for suspected tumor because of unilateral hematuria associated with an abnormal intravenous pyelogram Priapism has been reported in a number of patients it sometimes persists from 10 to 36 days and is often followed by complete impotence[15]

Nearly all patients with sickle cell anemia at one time or another complain of gastrointestinal symptoms such as anorexia nausea vomiting or jaundice Severe pain may occur in any part of the abdomen and at times it is difficult to decide whether or not surgical intervention is indicated Such pains which generally occur in episodes of "crisis may be due to infarction of the spleen or some other organ or may be related to cholelithiasis which has been reported to have an incidence of 25 per cent The abdominal pain is sometimes extremely severe At times it persists over a period of several days despite narcotics and other measures usually

employed in the relief of pain and then disappears completely without apparent residual damage. Severe abdominal pains have been attributed to development of infarcts in the vertebrae which may not become apparent on a roentgenogram until several weeks after the pain has subsided.[17]

A wide variety of central nervous system manifestations has been reported in patients of all ages.[69] These include hemiplegia, aphasia, dysphagia, nystagmus, drowsiness, coma, headache, convulsions and stiff neck. Electroencephalographic abnormalities are common in children.[9] *Evidence of the neurologic involvement may subside completely, but some* patients die during these episodes and others develop a spastic paralysis that persists. The lesions in the central nervous system are presumed to be the *result of vascular accidents or circulatory disturbances of a transient* nature.

Sickle cell anemia is often accompanied by symptoms or signs referable to the joints and skeleton. Pain in the joints is common, and when there is an associated heart murmur rheumatic fever may be suggested. However, in sickle cell anemia the pain is not usually severe and actual swelling of the joints is rare. Skeletal abnormalities are usually demonstrable only by roent-



Figure 50 Roentgenogram of the skull of a patient with sickle cell anemia showing the "hair on end" appearance.

genograms the commonest manifestations are "fish tailing" of the vertebral bodies and radiolucent areas of necrosis in these or other bones [149, 218] The "hair on end" appearance of the skull sometimes occurs but is not common (Fig 30) Other abnormalities that can be demonstrated radiologically in some patients are gallstones pulmonary infarcts and cardiac enlargement

LABORATORY EXAMINATION

In the usual patient with sickle cell anemia the hemoglobin and erythrocyte count are reduced to about half the normal values The anemia is normocytic and normochromic unless complicating factors are present. On the stained smear of peripheral blood a few sickled forms may be seen but the most prominent features are marked anisocytosis poikilocytosis and the presence of target cells nucleated red cells are often present (Fig 31) The leukocyte count is usually between 12 000 and 16 000 per cu mm the reported counts have ranged from 4000 to 69 000 per cu mm [9 —4] The platelet count is usually normal but counts over 1 000 000 per cu mm sometimes occur

The laboratory evidences of a hemolytic process are found unless an



Figure 31 Erythrocytes in sickle cell anemia Wright's stain
(Courtesy of Dr B E Washburn and the Virginia Medical Monthly vol 15 1911)

aplastic phase is present The reticulocyte count is often over 20 per cent unless it is depressed by some associated disease The level of the indirect reacting bilirubin in the serum is increased but it usually does not exceed 3 0 mg per 100 ml unless there is some associated hepatic or biliary complication Fecal urobilinogen excretion is increased and average daily excretions between 200 and 750 mg have been reported [6, 7] The daily fecal urobilinogen excretion varies spontaneously and decreases after transfusions of whole blood The plasma iron level is normal or elevated and the latent iron binding capacity is normal [1] The free erythrocyte protoporphyrin is usually elevated in a group of patients studied by the authors serial determinations disclosed unexplained variations that appeared to be independent of the reticulocyte count the degree of anemia or the presence of infection [18]

Examination of the bone marrow usually reveals erythrocytic hyperplasia of the normoblastic type associated with hyperplasia of the myeloid cells Acute transient marrow aplasia has been reported during an attack of pneumonia [39] Sickling of the adult erythrocytes and of the nucleated red cells may be observed in material obtained from the marrow Long filamentous erythrocytes are observed more often in the marrow preparations than in preparations made from the peripheral blood

The sedimentation rate in patients whose red cells sickle is not increased as much as is usually the case in patients with a comparable degree of anemia Prolonged stasis of venous blood causes an increased retardation of the sedimentation rate [1] Exposure of a sample of blood to carbon dioxide gas will reduce the sedimentation rate to less than 1 mm per hour while oxygenation of the blood will cause an increase in the sedimentation rate that amounts to from 23 to 70 mm per hour [36]

The erythrocytes of sickle cell anemia are abnormally resistant to hypotonic saline in one series the average figure for beginning hemolysis was 0 35 per cent and for completion was 0 22 per cent [136] Some of the cells are not hemolyzed in distilled water [187] The mechanical fragility of the cells is normal when they are in the discoid form but becomes increased if sickling is produced by exposure to carbon dioxide [18]

The characteristic sickling phenomenon can be demonstrated in most sealed cover slip preparations that were first introduced by Emmel [91] The undesirable features of the cover slip method are the time required for the sickling to take place even in patients with sickle cell anemia, and the occurrence of false negative results At least fourteen other tests that produce sickling have been described [1] The sodium bisulfite method has proved to be satisfactory in our experience [211] The use of hemoglobin electrophoresis as performed by Smith and Conley [31] has proved to be helpful in diagnosing many patients with sickle cell anemia It is particularly helpful in distinguishing patients with sickle cell anemia from those who have the sickle cell trait combined with some unrelated anemia, and from those patients who are heterozygous for two abnormal hemoglobins

DIFFERENTIAL DIAGNOSIS

There is usually no difficulty in establishing the diagnosis of sickle cell anemia if the possibility is considered A positive test for sickling indicates that the individual has either the heterozygous sickling trait or sickle cell anemia The demonstration of the sickling phenomenon in a patient who has anemia does not establish the diagnosis of sickle cell anemia because the sickle cell trait is common in Negroes who may have anemia from almost any cause An incorrect diagnosis of sickle cell anemia may be made in patients with sickle cell trait who have chronic blood loss or pernicious anemia unless proper studies are carried out Unless the patient has been transfused recently the diagnosis of sickle cell anemia can nearly always be established or excluded by hemoglobin electrophoresis The discovery of a relatively mild degree of anemia associated with a positive sickling test in a patient who has significant splenomegaly should arouse the suspicion that the patient has a combination of sickle cell trait plus some other hemoglobin abnormality such as hemoglobin C rather than sickle cell anemia Hemoglobin electrophoresis is the most valuable method of distinguishing sickle cell anemia from sickle cell hemoglobin C disease and other disorders that are due to the occurrence of sickle cell hemoglobin with some other abnormal hemoglobin

If the diagnosis of sickle cell anemia is not considered the disease may be mistaken for acute rheumatic fever when the patient presents with anemia joint complaints fever cardiac enlargement and cardiac murmurs The absence of the severe pains swelling and tenderness of the joints that are usually seen in rheumatic fever may help to distinguish between these two diseases In other patients with signs of acute intra abdominal disease it may be difficult to make the correct diagnosis because patients with sickle cell anemia sometimes develop acute surgical emergencies just as any other patients do but also they develop signs of acute intra abdominal disease as the result of sickle cell anemia alone Both possibilities must be considered when the patient is seen and the decision must be made on the basis of the evidence available at that time Sickle cell anemia must be differentiated from other diseases that cause ulcers of the extremities and from those that produce symptoms or signs of involvement of the central nervous system Sickle cell anemia which has been called the great masquerader [36] has been confused at times with rheumatoid arthritis osteomyelitis poliomyelitis meningitis cerebrovascular accident infectious hepatitis and other diseases [1]

COURSE AND PROGNOSIS

Sickle cell anemia is a serious disorder and few patients survive the third decade Many patients die during childhood Death comes most often from infections such as pneumonia and chronic pyelonephritis with renal failure

Other causes of death are heart failure shock with abdominal crisis complications in the central nervous system tuberculosis and sarcoidosis

The occurrence of pregnancy has been considered a serious matter in the patients with sickle cell anemia who reach the childbearing age In one review of the subject it was found that spontaneous abortion occurred in 13 per cent stillbirths occurred in 17 per cent and another 12 per cent of the children died soon after delivery [15] The maternal mortality rate seems to be between 10 and 20 per cent because of the occurrence of infection heart failure pulmonary embolism uremia shock and cerebral vascular accidents The occurrence of toxemia of pregnancy is common More recently it has been suggested that pregnancy is a more serious problem in sickle cell anemia–hemoglobin C disease than it is in sickle cell anemia [3]

In some patients with sickle cell anemia physical development is greatly retarded and hypogonadism occurs (Fig 32) In other patients the growth and development seem to follow a normal pattern The reason for these differences in patients with sickle cell anemia is not apparent but the differences in development are not due solely to differences in the severity of the anemia [16]



Figure 32 Body habitus of the type seen frequently in sickle cell anemia

TREATMENT OF SICKLE CELL ANEMIA

The treatment of sickle cell anemia is generally unsatisfactory Transfusions are the only means of returning the blood to normal levels that are available at present Transfusions are not used to maintain the blood in the normal range because patients with this disease adjust very well to their

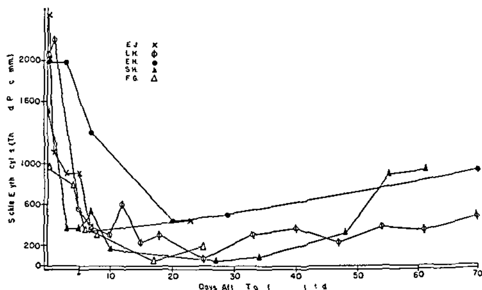


Figure 33 The effect of transfusion on the number of circulating sickle cells in sickle cell anemia (From Donegan MacIlwaine and Leavell Am J Med vol 17 1954)

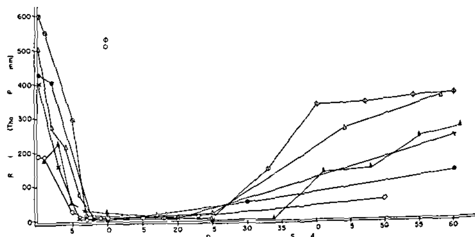


Figure 34 The effect of transfusion on the reticulocyte count in sickle cell anemia (From Donegan MacIlwaine and Leavell Am J Med vol 17 1954)

lowered hemoglobin levels Transfusions are valuable during periods of aplastic crisis abdominal crisis and at any other time when the patient is severely ill It is possible that they also may be advantageous in carrying patients through pregnancy or operations because multiple transfusions depress erythropoiesis and reduce the number and percentage of sickle cells in the peripheral circulation (Figs 33 and 34) [90]

Splenectomy is sometimes beneficial in children The beneficial effect seems to be related to the size of the spleen [84] In most adults with the disease the spleen is markedly reduced in size probably because of repeated infarctions and splenectomy has little place in the treatment

Various measures have been used in an effort to reduce the sickling and thereby relieve or reduce the severity of the clinical symptoms in these patients In this category are cortisone ACTH benzazoline (Priscoline) sodium bicarbonate and acetazolamide (Diamox) None of these agents can be considered to be of proven value at the present time

Patients may appear critically ill during episodes of "crisis" At such times blood transfusions antibiotics and measures to relieve pain are necessary The short term use of antibiotics appears justified even though the presence of an infection cannot be established because these episodes are so often preceded by an infection

ILLUSTRATIVE CASE

This patient a male Negro was first admitted to the hospital in July 1946 at the age of 12 because of fever cough and pleuritic pain of one week s duration For some years he had been troubled with recurring chronic ulcers in the lower tibial region A brother 10 years of age had a similar illness characterized by recurring attacks of fever and chronic ulceration of the legs One sister had died ten days previously of unknown cause The patient s father mother and four siblings were alive and well

Physical Examination Temperature 105 pulse 120 respirations 26 and blood pressure 105/55 The patient was alert thin and appeared acutely ill The veins in both fundi were extremely tortuous Rhonchi were heard throughout both lungs and moist rales were present in the lower portion of both lungs The heart did not appear enlarged but a systolic murmur was heard over the entire precordium Above the left internal malleolus there was an ulcer 4 cm. in diameter

Laboratory Examination Admission blood studies hb 4 0 gm per 100 ml rbc 1 4 mil per cu mm wbc 22 000 per cu mm retics 0 1 per cent The leukocyte differential count was normal but a few sickled erythrocytes and many target cells were present on the fixed stained smear A moist coverslip preparation was positive for sickling The icterus index was 6 units The urine had a specific gravity of 1 010 and was otherwise normal A chest roentgenogram showed increased bronchial markings and an infiltrate in the left lower lobe

Treatment and Course A diagnosis of sickle cell anemia with pneu

monia was made The patient was given blood transfusions and penicillin and made an uneventful recovery Before discharge from the hospital on the sixteenth day the leukocyte count had fallen to 11 000 per cu mm and the reticulocyte count had risen to 5 4 per cent despite numerous transfusions

This patient was observed for eight years during which time his course was characterized by chronic ill health and exacerbations of acute illnesses The erythrocyte count was usually in the range of 1 5 to 2 0 mil per cu mm and the reticulocyte count was usually 25 or 30 per cent On several occasions the fecal urobilinogen excretion was more than 1000 mg in 24 hours The growth and development were subnormal The extremities were long and thin the trunk was short and no secondary sex characteristics developed At the age of 20 he weighed but 80 pounds and appeared to be about 10 years of age He developed repeated respiratory infections and was hospitalized on four occasions because of pneumonia In some of these attacks the reticulocyte count was 0 1 per cent and the serum bilirubin was less than 1 0 mg per 100 ml in others the reticulocyte count was 18 per cent and the serum bilirubin was 3 0 mg per 100 ml During this time repeated roentgenograms revealed progressive enlargement of the heart In December 1954 when he was 21 years old this patient was hospitalized with congestive heart failure and severe anemia He failed to respond to the usual measures and died in acute heart failure

Postmortem Postmortem examination revealed malnutrition and generalized underdeveloped musculature The over all length of the body was 169 cm 89 cm of which was formed by the lower extremities The testes appeared to be prepubertal and a section through the prostate gland disclosed only a few glands that appeared immature The heart was moderately hypertrophied and weighed 370 grams The right atrium and ventricle were markedly dilated The pulmonary artery was greatly dilated from its point of origin but no thrombi were demonstrated in the larger branches Microscopic examination of the lungs disclosed emphysema thickening of the walls of the smaller pulmonary arteries that produced complete obliteration of the lumen in some areas and multiple small granulomatous lesions The lymph nodes from various parts of the body were enlarged and contained innumerable discrete epithelioid cell granulomas These granulomatous lesions were diagnosed as Boeck s sarcoid The liver was enlarged weighed 2250 grams and contained large amounts of hemosiderin The spleen was scarred and contracted and weighed only 28 grams The bone marrow was hyperplastic

Comment Many of the commonest features of sickle cell anemia were evident in this patient This young Negro had a severe hemolytic anemia chronic ill health and retarded physical development The most striking abnormalities in the physical examinations were the disproportion between the size of the trunk and the length of the extremities the long tapering fingers hypogonadism pallor of the mucous membranes evidence of ulceration in the lower legs the systolic murmur over the precordium and the tortuous "corkscrew" appearance of the retinal veins At various times the

patient suffered from leg ulcers, abdominal pains, and attacks of pneumonia. During his first admission he apparently had an episode of "aplastic crisis," although it was not recognized as such at that time; other attacks of pneumonia of equal severity did not produce such depression of the bone marrow. He died at the age of 21 of heart failure associated with cor pulmonale. Autopsy disclosed obstruction in the pulmonary circulation and cardiac hypertrophy but no evidence of valvular disease. The cardiac hypertrophy involved both sides of the heart and may have been due partly to the chronic severe anemia. As is often the case, the spleen was scarred and smaller than normal. The presence of Boeck's sarcoid that was demonstrated at the post-mortem examination was not suspected ante mortem.

Electrophoresis of blood from this patient and his brother, who had a strikingly similar physical habitus, revealed the presence of only hemoglobin S (homozygous SS). Study of the parents, both of whom had positive tests for sickling without anemia, showed the presence of both hemoglobin A and hemoglobin S in each (heterozygous AS).

CLINICAL SYNDROMES (OTHER THAN SICKLE CELL ANEMIA AND THALASSEMIA) ASSOCIATED WITH ABNORMAL HEMOGLOBINS

HOMOZYGOUS HEMOGLOBIN C DISEASE

Hemoglobin C was first described by Itano and Neel.[47] Instances of homozygous hemoglobin C disease have been reported by a number of observers.[13, 23 … 6, 28 … 61] The disease occurs almost exclusively in Negroes, where the incidence of hemoglobin C trait has been found to be about 2 or 3 per cent. The patients that have been reported have presented a fairly uniform clinical picture characterized by splenomegaly, mild or moderately severe hemolytic anemia, and recurrent jaundice. A report of the disease in one patient who was 74 years of age indicates that the disorder is consistent with a normal life span.[61]

The hemoglobin values in the reported cases have varied from 8.7 to 12.5 gm. per 100 ml.[60] The most prominent feature of the stained smear is the presence of numerous target cells that often constitute from 50 to 100 per cent of the erythrocytes. The osmotic fragility of the red cells is decreased. Hemoglobin electrophoresis discloses the presence of only hemoglobin C. Mild reticulocytosis is usually present. The erythrocytes have a decreased



Figure 35 Target cells and hemoglobin crystals in an isotonic saline suspension of erythrocytes from the peripheral blood of a patient with homozygous hemoglobin C disease after splenectomy Note persistence of the cellular membrane about the hemoglobin crystal in the center of the picture

life span and the fecal urobilinogen excretion is increased [60] Intraerythrocytic hemoglobin crystals have been observed in patients who have undergone splenectomy (Fig 35) [43] [61]

SICKLE CELL-HEMOGLOBIN C DISEASE

Hemoglobin C was first recognized as a new hemoglobin in a study of patients who were diagnosed as atypical sickle cell anemia but instead proved to have sickle cell-hemoglobin C disease [4] The presence of both hemoglobin C and hemoglobin S was demonstrated by electrophoresis In the reported patients the major portion of the hemoglobin has been of the C type [63]

The clinical features of this disease have been described by several authors [48] [6] The disease is much milder than sickle cell anemia but severe episodes of crisis occur in some patients pregnancy may be associated with serious complications [6] In adults the spleen is usually considerably enlarged in contrast to sickle cell anemia where a small spleen is an almost constant feature The anemia is usually mild or moderate in degree and the hematocrit and hemoglobin values range from 70 to 100 per cent of normal except during episodes of crisis The reticulocyte count is normal or slightly increased [6] On fixed stained smears of the peripheral blood from 50 to 100 per cent of the erythrocytes appear as target cells [6] Sickling can be demonstrated in moist preparations The osmotic fragility of the erythrocytes is

decreased [63] The diagnosis is established by electrophoresis which reveals the presence of both hemoglobin S and hemoglobin C As in other hemolytic anemias the survival time of the erythrocytes is decreased fecal urobilinogen excretion is increased and the erythropoietic tissue in the marrow undergoes the normoblastic type of hyperplasia [63]

THALASSEMIA-SICKLE CELL DISEASE

Thalassemia-sickle cell disease probably was first described by Silvestroni and Bianco in 1946 before electrophoretic studies of hemoglobin were made The vast majority of the patients with this disease are Caucasians of Greek or Italian stock [63] It has been possible to demonstrate that the patient with this disease is a double heterozygote for the two abnormal genes present in the parents [9]

The disease resembles sickle cell anemia but the severity of the clinical manifestations varies greatly from one patient to another The hematologic characteristics are microcytic hypochromic anemia erythrocytic sickling in moist preparations and the presence of numerous target cells on the fixed smear [63] Reticulocytosis is usually present Patients differ in the severity of the anemia and in the degree of the splenomegaly

SICKLE CELL-HEMOGLOBIN D DISEASE

Hemoglobin D was described first by Itano [4] It has been found to be more common among non Negroes in some parts of the world but is more common in Negroes than in Caucasians in the United States [7] The incidence in Negroes in the United States has been reported to be 1 1000 [7] and 4 1000 [30]

Sickle cell-hemoglobin D disease has been the subject of several reports [7] [4] The patients who have this disorder are normally developed often remain asymptomatic for long periods and may have little or no anemia [7] Attacks that resemble mild episodes of sickle cell crisis occur Differentiation of this disorder from sickle cell anemia may be difficult because in both diseases sickling occurs in preparations of fresh blood and filter paper electrophoresis at pH 8 6 shows only a single concentration peak that has the mobility of S hemoglobin In some instances the differentiation can be made by comparing the solubilities of the reduced forms of the hemoglobin because the solubility of reduced hemoglobin D is normal while that of reduced hemoglobin S is decreased [4] Differential solubility studies may not be conclusive and in such circumstances electrophoresis on agar at pH 6 2 may be utilized to differentiate the two hemoglobins

HEMOGLOBIN H DISEASE

Hemoglobin H was described first by Rigas Koler and Osgood [1] The proportion of the abnormal hemoglobin varies from 10 to 35 per cent in

patients with hemoglobin H disease [38] The disorder is characterized by mild hemolytic anemia (hb 10 0 11 0 gm per 100 ml) mild reticulocytosis slight reduction in the survival time of the erythrocytes slight increase in resistance to hypotonic saline and normal or slightly increased levels of serum bili rubin Although hemoglobin H disease resembles thalassemia minor clini cally in hemoglobin H disease target cells are absent and Hb A_2 is not increased [-38] It has been suggested that the gene for hemoglobin H does not find expression in the phenotype unless it is associated with a thalassemia gene [49] However the absence of hemoglobin A in the families of patients with hemoglobin H disease has been reported [-44]

LESS COMMON SYNDROMES

Thalassemia C disease and thalassemia E disease are similar clinically and hematologically Splenomegaly mild hypochromic anemia target cells and increased resistance to hypotonic saline occur in both diseases [41 4 6 -63]

Hemoglobin E was described independently in 1954 by Itano Bergren and Sturgeon in the United States [16] and by Chernoff A Minnich Nana korn and Chernoff R in Thailand where it appears to be much more common [40] Hemoglobin E migrates slightly faster than hemoglobin C on paper at pH 8 6 The clinical features of homozygous hemoglobin E disease which has been found in patients in Thailand are similar to those seen in hemoglobin C disease Both are characterized by mild hemolytic anemia splenomegaly arthralgia and large numbers of normochromic target cells [41 63] The occurrence of hemoglobin E trait is associated with no disability [6]

Homozygous hemoglobin D disease has been reported in a 40 year old Negro This patient had erythrocyte counts that varied from 5 5 to 6 5 mil per cu mm and hemoglobin values that ranged from 12 5 to 13 5 gm per 100 ml The MCV was 67 cu microns from 60 to 70 per cent of the erythrocytes were target cells A slight decrease in survival time of the erythrocytes was demonstrated [4]

THALASSEMIA

Synonyms Mediterranean Anemia Cooley s Anemia Hereditary Leptocytosis Erythroblastic Anemia

Thalassemia or "Cooleys anemia" was first recognized as a clinical entity by Cooley and Lee who described five cases of this disorder in 1925 [67] A

similar but much less severe disease found in adolescents and adults was reported in 1940 by Wintrobe, Matthews, Pollack, and Dobyns.[8] As a result of studies on the genetics of the disease Valentine and Neel concluded that these disorders were related and suggested that the term "thalassemia major" be used *to denote the severe form of the disease which appeared to be the* homozygous condition and the name "thalassemia minor" be given to the *milder form which appeared to be the heterozygous state.*[7]

The numerous hematologic and genetic studies that have been carried out in recent years in families with thalassemia, with and without other hemoglobinopathies, indicate that the problem is a complex one. The difficulties that are encountered in classifying and recognizing all the varieties of thalassemia have been discussed.[6, 1] Although thalassemia is associated with three abnormalities of hemoglobin synthesis—the presence of hemoglobin F, the suppression of hemoglobin A production, and an increase in the proportion of hemoglobin A—none of these abnormalities is invariably present. Hemoglobin F occurs regularly in patients with thalassemia major and it occurs often but not invariably in heterozygotes.[1] The occurrence of the suppression of hemoglobin A production without the presence of any associated morphologic features of thalassemia has been reported. Such cases *have been recognized by examination of other members of the family.* Hemoglobin A has been found in nearly all heterozygotes and is valuable as confirmatory evidence in the diagnosis, but the failure to find any increase in hemoglobin A_2 does not exclude thalassemia.[68] It is also recognized that all patients with thalassemia do not conform to the pattern of a severe disease in the homozygous state or a mild disorder in the heterozygous condition; intermediate forms occur. For these reasons it appears that there is more than one kind of thalassemia and probably more than one kind of thalassemia or thalassemia-like gene.[68]

MECHANISM OF ANEMIA

The anemia in thalassemia is classified as the intracorpuscular type of hemolytic anemia because erythrocytes from patients with the severe form of the disorder have a shortened survival time when transfused into normal recipients while normal erythrocytes survive for the normal span when transfused into patients with the disease.[69] Transfusion studies have also shown that the life span of the erythrocytes of patients with the milder or trait forms of the disorder have a normal survival time. The presence of an additional extracorpuscular mechanism has been demonstrated in some patients who have marked splenomegaly and have received numerous transfusions.[3] The nature of the mechanism that is actually responsible for the increased hemolysis is unknown. The shortened life span of the cells apparently is not due solely to the morphology of the erythrocytes, because normal survival of the red cells has been demonstrated in some patients who have similar morphologic abnormalities, such as leptocytic erythrocytes and hypochromic microcytes.[69, 9] It is not clear that the presence of hemoglobin

F is of any importance in the pathogenesis of the disease because there appears to be no correlation in the individual patient between the amount of this hemoglobin and the severity of the clinical manifestations.

The shortened survival time of the erythrocytes in thalassemia, slightly less than half that of normal cells,[63-66] is nearer normal than the survival time of the erythrocytes of patients with hereditary spherocytosis and sickle cell anemia. The association of a moderate reduction in the survival time of the cells with a severe degree of anemia in some patients strongly suggests that erythropoiesis is defective.[68] Other observations have been presented as evidence of such a defect. These include the failure to find any correlation between the degree of hyperbilirubinemia and the life span of the erythrocytes and the variation in the reticulocyte counts. The latter suggests that the severe form of the anemia is related primarily to the state of the bone marrow function, which has been reported to undergo periodic depression and acceleration of erythropoiesis. In addition, histologic study of the bone marrow has been interpreted as indicating defective maturation because the maturation of the nuclei appeared to be normal while the cytoplasm remained basophilic. The occurrence of a hypochromic type of anemia despite normal iron stores indicates that erythropoiesis is also associated with some defect in iron metabolism.[68]

CLINICAL MANIFESTATIONS

Thalassemia occurs mainly in families of Mediterranean origin, particularly those from Italy, Greece, Sicily, and Syria, but it also is seen in Negroes and in Southeast Asia.[3] In a large Italian population in Rochester, N. Y., the incidence of thalassemia minor has been estimated at one in 25 persons and of thalassemia major as one in 2368 births.[4]

Thalassemia minor, the heterozygous form of the disease, is largely asymptomatic. It is the type seen most often in adults. In its mildest form, which may be found by chance or in the study of the family of a patient with the major form of the disease, the only apparent abnormality in the routine laboratory tests may be the presence of leptocytosis. Other patients with this mild form of the disease have more pronounced hypochromic microcytosis and mild reductions in hemoglobin and hematocrit associated with an elevated red cell count. In patients with thalassemia minor appropriate studies often demonstrate an increased osmotic resistance of the red cells associated with increases in the hemoglobin F and hemoglobin A. Splenomegaly, slight reticulocytosis, and mild icterus are sometimes present. The occurrence of "intermediate" forms of thalassemia with moderately severe anemia is not well understood. It appears likely that many instances of the intermediate form are in reality patients in whom the heterozygous thalassemia gene is associated with some other unrecognized hemoglobin abnormality.

Thalassemia major, the homozygous form of the disease, is a severe disorder that generally becomes manifest early in childhood, nearly always in the first decade. Pallor and easy fatigue often appear in the first few years

of life As the disease progresses the diagnosis may be suggested by the patient's appearance The head often appears unduly large The prominence of the malar eminences associated with depression of the bridge of the nose and the muddy yellowish color of the skin was termed "Mongolian" by Cooley and Lee[6] Marked splenomegaly and hepatomegaly are usually present The abdomen often protrudes because the spleen may fill the left half of the abdominal cavity Chronic leg ulcers sometimes occur but are not common[7] Evidence of abnormalities of the cardiovascular system in the form of cardiac enlargement edema pleural effusions and ascites is not unusual. Skeletal defects may be prominent but they are not pathognomonic of thalassemia The changes in the skull consist of a thinning of the inner and outer table associated with a widening of the diploe which may contain prominent vertical striations that produce the "hair on end" appearance of the skull Alterations in the long bones which are less striking consist of widening of the medullary canals thinning of the cortices osteoporosis and heavy trabeculations near the ends of the shaft

Thalassemia major must be considered a fatal disease at present because the majority of patients do not survive to adult life The prognosis appears worse if the disease becomes manifest early in life Death is usually caused by an intercurrent infection

LABORATORY EXAMINATION

Thalassemia major is characterized by a microcytic hypochromic type of anemia[6] The anemia is usually severe but the erythrocyte count may range from 1 0 mil per cu mm to 4 0 mil per cu mm The MCV is often less than 60 to 70 cu microns[6,9] The reduction in the MCHC is not as pronounced as the decrease in the MCH Stained films of the peripheral blood show a severe degree of poikilocytosis anisocytosis and polychromatophilia The erythrocytes appear small and hypochromic Many stippled cells and "target" cells are present Nucleated red cells which are almost invariably present are often numerous and sometimes outnumber the leukocytes Reticulocytosis is usually present and is accompanied by normoblastic hyperplasia in the bone marrow Leukocyte counts in the range of 10 000 to 25 000 per cu mm are common and immature forms sometimes appear in the peripheral blood The platelet count is generally normal

In thalassemia minor the erythrocyte count is often normal or increased even though the hemoglobin and hematocrit are reduced In some such cases there may be a mild reticulocytosis and slight hyperbilirubinemia Target cells anisocytosis poikilocytosis polychromatophilia, and stippling are evident on the blood smear and are much more prominent than one would expect from the degree of anemia that is present

Other features common to most hemolytic anemias are usually present in thalassemia major These are elevation of the indirect reacting bilirubin in the serum increased excretion of urobilinogen in the feces and urine elevation of the serum iron, and decrease in the latent iron binding capacity

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CLINICAL MANIFESTATIONS

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Thalassemia major the homozygous form of the disease is a severe disorder that generally becomes manifest early in childhood nearly always in the first decade Pallor and easy fatigue often appear in the first few years

anemia but this form of therapy is considered as a symptomatic measure Transfusions are given when the symptoms warrant but are not used to maintain the hemoglobin in the normal range Many patients adjust fairly well to their low hemoglobin levels for considerable periods and unnecessary transfusions increase the danger of hemosiderosis The administration of iron pyridoxine folic acid vitamin B_1 and crude liver extract produces no improvement in the anemia and the needless administration of iron may promote the development of hemosiderosis When the spleen is greatly enlarged and associated with an extracorpuscular hemolytic mechanism splenectomy may be beneficial even though it does not completely correct the anemia

ILLUSTRATIVE CASE

A 60 year old white housewife who was largely asymptomatic was admitted for evaluation of anemia She stated that her hematocrit had been about 35 per cent for a number of years and previous examinations had disclosed no cause for her anemia The physical examination was considered to be within the limits of normal for a person of her age

Laboratory Examinations Hct 36 per cent rbc 5 0 mil per cu mm hb 10 6 gm per 100 ml (MCV 72 cubic microns MCH 21 $\mu\mu$g MCHC 28 per cent) reticulocyte count 2 4 per cent leukocyte 6100 per cu mm Differential count of the leukocytes was normal Study of the stained smear disclosed hypochromic microcytosis anisocytosis and poikilocytosis A moderate number of target cells and a few stippled cells were present Total serum bilirubin was 0 4 mg per 100 ml Osmotic fragility test patient s cells initial 0 42 per cent complete 0 30 per cent control initial 0 45 per cent complete 0 30 per cent Examination of the bone marrow disclosed a hypercellular marrow with erythroid hyperplasia of the normoblastic type The granulocytic series and megakaryocytic series appeared normal and the M E ratio was 1 1 Hemoglobin electrophoresis on filter paper showed only hemoglobin A The alkali denaturation test disclosed 10 per cent of the hemoglobin to be alkali resistant presumably hemoglobin F The serum iron level and the level of the unsaturated iron binding capacity were within limits of normal.

Familial Incidence A diagnosis of thalassemia minor was made The patient s 28 year old daughter who also gave a history of unexplained anemia, was studied and the laboratory findings were almost identical with those found in the mother s blood The hemoglobin was 10 3 grams per 100 ml rbc 5 1 mil per cu mm hct 38 per cent The reticulocyte count was 1 5 per cent. Leukocyte count and differential leukocyte counts were normal Study of the stained smear disclosed anisocytosis poikilocytosis target cells and stippled cells The serum iron level was 112 μg per 100 ml The osmotic fragility test patient s cells initial hemolysis 0.39 per cent, completed 0 21 per cent control beginning hemolysis 0 46 per cent, complete 0 32 per cent. This patient (the daughter) was followed for several months during which

In addition the erythrocytes are abnormally resistant to hemolysis in hypotonic saline hemolysis may not be complete at 0 2 per cent saline or even in distilled water [64]

One of the characteristic hematologic features of thalassemia is the presence of large amounts of hemoglobin F Hemoglobin of this type constitutes from 50 to more than 90 per cent of the hemoglobin in some patients with thalassemia major [1] *It may or may not be demonstrable in the milder* syndromes but often amounts to 20 to 40 per cent of the hemoglobin in the intermediate forms The alkali resistant hemoglobin can be demonstrated by the alkali denaturation technique The presence of hemoglobin A usually in increased amounts is found in nearly all patients with thalassemia minor when hemoglobin electrophoresis is performed on starch slabs [71]

DIAGNOSIS

The appearance of the typical patient with thalassemia major may suggest the diagnosis In thalassemia major the diagnosis usually is not difficult because of the evident hepatosplenomegaly the presence of anisopoikilocytosis target cells and normoblasts on the blood smear evidence of hemolysis and the presence of increased amounts of hemoglobin F

*Although the diagnosis of thalassemia major is rarely difficult the distinc*tion between the milder forms of thalassemia and other types of hemolytic anemia and hemoglobinopathies is not always easy The occurrence of hypochromic microcytosis—with or without anemia—target cells nucleated red cells and stippled cells in the peripheral blood of a young person with *hepatosplenomegaly suggests the diagnosis At other times* the presenting problem may be a hypochromic microcytic anemia that must be distinguished from iron deficiency In doubtful cases either the determination of the plasma iron level which is low in iron deficiency and normal or high in thalassemia or an evaluation of iron stores in the bone marrow is helpful The finding of microcytic polycythemia in a patient is sometimes the first indication that the patient has thalassemia The demonstration of increased resistance of the erythrocytes in the hypotonic saline test supports the diagnosis The demonstration of an increase of hemoglobin A_2 by electrophoresis and the demonstration of increased amounts of hemoglobin F can be *considered as establishing the diagnosis The recognition of the presence of* a thalassemia gene in patients who have some other inherited hemoglobinopathy may be very difficult For example a person who is heterozygous for hemoglobin S and the thalassemia gene may appear to have sickle cell anemia clinically and show only hemoglobins S and F on electrophoresis because of the suppression of hemoglobin A [68] Studies of other members of the family may be necessary to establish the true nature of the disorder

THERAPY

Patients with thalassemia minor rarely need treatment *In thalassemia* major blood transfusions are the only effective means of correcting the

studied the incidence of the Rh factor in mothers and fathers of patients with erythroblastosis. They concluded that iso-immunization with Rh explained 91 per cent of the cases and thought that other factors were responsible for 9 per cent. They also concluded that the Rh factor was limited to the red cells and was not present in the saliva and other tissues.[94-96] In 1943 Mollison demonstrated that Rh positive red cells were eliminated rapidly after transfusion into infants with erythroblastosis during the first fourteen days of life while Rh negative cells were eliminated at the normal rate.[99] The value of exchange transfusion with Rh negative blood was demonstrated by Wallerstein in 1946.[343] The value of this form of treatment in preventing the most serious aspect of the disease, kernicterus, was shown in 1950 by Allen, Diamond and Vaughan,[82] who have made numerous other contributions to the understanding and management of the disease.

MECHANISM OF DISEASE

The anemia in erythroblastosis fetalis is hemolytic in type.[80, 85, 96, 99] The destruction of the fetal red cells is caused by the presence in the fetal circulation of iso-antibodies that are formed in the mother in response to a blood group antigen that the mother lacks. Agglutinogens A, B and D are strongly antigenic and the others are less so.[80] Allen and Diamond list 49 blood group factors that were known in July 1957 to be inherited characteristics. They state that A and B incompatibilities are responsible for about two-thirds of the cases of erythroblastosis fetalis, D (Rh) for about one-third and other blood factors for 2 or 3 per cent.[80] The occurrence of ABO hemolytic disease is virtually limited to group A or B offspring of group O mothers.[304] There is considerable individual difference in the ability to form antibodies and less than 10 per cent of the Rh negative mothers who have Rh positive husbands become sensitized; even if the husband is homozygous Rh positive (DD), the wife may have many children without becoming sensitized.[80]

The method of sensitization of the mother to the fetal red cells in the cases of Rh (D) incompatibilities is an intriguing problem. Although the administration of incompatible blood by transfusion or by intramuscular injection may be responsible for sensitization in women prior to pregnancy, the iso-antibody formation in mothers is usually the result of pregnancy. Fetal red cells may gain entrance to the mother's circulation during delivery. Allen and Diamond report that quantities of blood as small as 0.1 to 1.0 ml may be sufficient to produce sensitization and suggest that just as the normal kidney allows the passage of red cells into the urine, so the placenta may allow slow leakage of red cells into the mother's circulation.[80] As evidence to support this hypothesis they cite the observation that ABO incompatible fetuses almost never sensitize the mother to Rh, presumably because the fetal cells are destroyed too rapidly to act as efficient antigens. Also compatible with the hypothesis is the fact that the anti-Rh titer of the mother's blood falls after pregnancy and rises again during subsequent pregnancies with Rh positive fetuses.

time the erythrocyte count varied from 4 9 mil per cu mm to 6 1 mil per cu mm The fecal urobilinogen excretion was measured on several 4 day periods and was found to be normal each time An Ashby survival time study patient to normal recipient revealed that the patients cells survived for a normal time 120 days

Comment These patients demonstrated the occurrence of thalassemia minor or the intermediate form in two generations of the same family The mild hypochromic microcytic type of anemia associated with erythrocyte counts that reached polycythemic levels at times the appearance of target cells and other abnormal forms in the peripheral blood the normal values for the serum iron the increased resistance of the erythrocytes to hypotonic saline the presence of fetal hemoglobin are common findings in such patients The homozygous type of disease thalassemia major becomes evident early in life and is manifested by a severe hypochromic microcytic hemolytic anemia associated with a marked diminution in the survival time of the erythrocytes In thalassemia minor hypochromic microcytes occur but anemia is mild or absent and the survival time of the erythrocytes is normal The anemia can be distinguished from iron deficiency by the normal values of the serum iron and latent iron binding capacity

ERYTHROBLASTOSIS FETALIS

Synonyms Hydrops Fetalis, Icterus Gravis Neonatorum, Anemia of the Newborn Hemolytic Disease of the Newborn Caused by Rh and ABO Incompatibility

HISTORY

The term erythroblastosis was first used by Rautman in 1912[30] In 1932 Diamond Blackfan and Baty reviewed 2 cases with universal edema 12 with icterus gravis neonatorum and 6 with anemia of the newborn and concluded that these three disorders were closely related and dependent on the same underlying process [58] In 1939 Levine and Stetson reported the case of a group "O 25 year old secundipara who delivered a macerated stillborn fetus and suffered a severe hemolytic reaction when transfused with her husbands group O blood the mother was shown to have an iso agglutinin independent of M N and P blood factors that reacted with the blood of 45 of 50 group O donors [95] In 1941 Landsteiner and Wiener reported their studies of the Rh agglutinogen in human blood these indicated that the factor is inherited as a Mendelian dominant independent of the blood groups and factors M and N [93] In the same year Levine and coworkers

studied the incidence of the Rh factor in mothers and fathers of patients with erythroblastosis They concluded that iso immunization with Rh explained 91 per cent of the cases and thought that other factors were responsible for 9 per cent They also concluded that the Rh factor was limited to the red cells and was not present in the saliva and other tissues [94, 96] In 1943 Mollison demonstrated that Rh positive red cells were eliminated rapidly after transfusion into infants with erythroblastosis during the first fourteen days of life while Rh negative cells were eliminated at the normal rate [9] The value of exchange transfusion with Rh negative blood was demonstrated by Wallerstein in 1946 [93] The value of this form of treatment in preventing the most serious aspect of the disease kernicterus was shown in 1950 by Allen Diamond and Vaughan [5] who have made numerous other contributions to the understanding and management of the disease

MECHANISM OF DISEASE

The anemia in erythroblastosis fetalis is hemolytic in type [50, 96, 9] The destruction of the fetal red cells is caused by the presence in the fetal circulation of iso antibodies that are formed in the mother in response to a blood group antigen that the mother lacks Agglutinogens A B and D are strongly antigenic and the others are less so [50] Allen and Diamond list 49 blood group factors that were known in July 1957 to be inherited characteristics They state that A and B incompatibilities are responsible for about two thirds of the cases of erythroblastosis fetalis D (Rh) for about one third, and other blood factors for 2 or 3 per cent The occurrence of ABO hemolytic disease is virtually limited to group A or B offspring of group O mothers [3, 4] There is considerable individual difference in the ability to form antibodies and less than 10 per cent of the Rh negative mothers who have Rh positive husbands become sensitized even if the husband is homozygous Rh positive (DD) the wife may have many children without becoming sensitized [50]

The method of sensitization of the mother to the fetal red cells in the cases of Rh (D) incompatibilities is an intriguing problem Although the administration of incompatible blood by transfusion or by intramuscular injection may be responsible for sensitization in women prior to pregnancy the iso-antibody formation in mothers is usually the result of pregnancy Fetal red cells may gain entrance to the mother s circulation during delivery Allen and Diamond report that quantities of blood as small as 0 1 to 1 0 ml may be sufficient to produce sensitization and suggest that just as the normal kidney allows the passage of red cells into the urine so the placenta may allow slow leakage of red cells into the mother s circulation [5] As evidence to support this hypothesis they cite the observation that ABO incompatible fetuses almost never sensitize the mother to Rh presumably because the fetal cells are destroyed too rapidly to act as efficient antigens Also compatible with the hypothesis is the fact that the anti Rh titer of the mother s blood falls after pregnancy and rises again during subsequent pregnancies with Rh positive fetuses

Edema which may be severe in babies with erythroblastosis is associated with an elevated venous pressure and is considered to be a manifestation of heart failure that is secondary to anemia [80, 93]

Kernicterus circumscribed areas of yellow coloring in the brain that involve the nuclear areas particularly is one of the most serious manifestations of erythroblastosis fetalis It causes death within seven days in 70 per cent of the babies who have this complication [91] The brain damage which is not *present at birth has been shown to be related to the level of the serum* bilirubin since kernicterus occurs in 50 per cent of babies whose level of the serum bilirubin is over 30 mg per 100 ml In newborn babies the ability of the liver to convert indirect reacting bilirubin (unconjugated bilirubin) to the direct reacting form (bilirubin glucuronide) for excretion is poorly developed the deficiency is even more marked in premature infants [83] Defects in the glucuronyl transferase and uridyldiphosphoglucose dehydrogenase activity that is necessary for the synthesis of bilirubin glucuronide have been demonstrated in the livers of fetal and newborn guinea pigs and support the hypothesis that the glucuronide conjugating mechanism is inadequately developed in human newborns and prematures When this mechanism is defective even though the level of serum bilirubin may not be greatly elevated at birth probably because the placenta in some way aids in the excretion in utero the level of the serum bilirubin rises rapidly after birth if hemolytic anemia is present Unconjugated bilirubin has been identified as the pigment present in the brain in infants dying with kernicterus That bilirubin is toxic to cells is supported by the findings that this pigment inhibits oxidative phosphorylation depresses respiration of liver and brain tissue and in vitro is an inhibitor of heme synthesis [79] Inhibition of heme synthesis in vivo might interfere with the formation of heme containing enzymes

It is also possible that other pigments may be important in kernicterus Fatalities from kernicterus have occurred in patients with serum bilirubin levels of from 1 to 6 mg per 100 ml in such patients the plasma has been shown to have increased levels of heme pigments with an absorption maximum in the range of 405 to 415 mμ which is different from the absorption maximum for bilirubin 460 mμ [9] It has been suggested that in patients with early kernicterus and relatively low serum *bilirubin levels methemalbumin* may interfere with the ability of the bilirubin to concentrate on the serum proteins [3, 1] Brown Zuelzer and Robinson have demonstrated that the serum bilirubin concentration is not always a gauge of the diffusible or total body bilirubin [4] If the bilirubin cannot bind with the protein in the vascular spaces the plasma level may be low even though bilirubin has diffused into the cells in appreciable quantities This shift may be of special importance in premature infants who have lower serum albumin levels than full term infants [57] Odell has also demonstrated that organic anions salicylate and sulfisoxazole can uncouple protein bound bilirubin and allow its passage through semipermeable membranes [61]

CLINICAL MANIFESTATIONS

Erythroblastosis is reported to occur in about one per cent of pregnancies[80] About one fifth of the affected infants die in utero or are born critically ill with anemia at the time of birth

Many erythroblastotic infants appear normal at birth Jaundice is said to be invariably absent at birth but it may appear within two or three hours and may increase rapidly thereafter unless treatment is instituted Petechial hemorrhages sometimes develop soon after birth Hepatomegaly and splenomegaly occur almost invariably in Rh disease When erythroblastosis is due to A or B incompatibility the spleen is often enlarged but to a lesser degree than in Rh disease Pulmonary edema pleural effusions ascites and edema may be manifestations of heart failure Kernicterus which usually becomes manifest late in the second day of life is rare in the absence of considerable jaundice[9, 91] Any infant with erythroblastosis should have frequent and careful neurologic examinations In the early stages kernicterus may be accompanied by no more than minimal neurologic signs that are easily missed When the kernicterus is more pronounced, the affected infant has a high pitched cry irritability tremor and an overactive but incomplete Moro reflex opisthotonos twitching convulsions and respiratory distress may also occur If the child has kernicterus and survives sequelae such as deafness mental retardation athetoid movements and spastic ataxia develop

LABORATORY EXAMINATION

Anemia is considered to be present if the hemoglobin level of the cord blood is less than 13 0 gm per 100 ml[98] if the hemoglobin level of the venous blood is less than 15 gm per 100 ml[80] or if the hemoglobin level of capillary blood is less than 16 0 gm per 100 ml on the first day[98] A hemoglobin level of less than 9 0 gm per 100 ml at birth indicates anemia of severe degree Reticulocytosis is usually present A high percentage of reticulocytes indicates a severe hemolytic process even if associated with a normal level of hemoglobin[5] Nucleated red cells are commonly present in the peripheral blood but may be absent in one third of the cases on the first day[98] Elevated leukocyte counts are the rule and have little prognostic value Thrombocytopenia often occurs in severe cases

The normal level of serum bilirubin on cord blood is considered to be from 0 8 to 2 6 mg per 100 ml[98] The level of indirect reacting serum bilirubin in newborn babies at 48 hours rarely exceeds 13 mg per 100 ml In erythroblastosis the level of the serum bilirubin may be normal at birth and rise rapidly thereafter For this reason frequent determinations are most important in the management of these patients

DIAGNOSIS

Erythroblastosis fetalis must be distinguished from other causes of anemia such as hereditary spherocytosis and hemorrhage and from other causes

of jaundice such as infectious hepatitis bacterial infections syphilis cytomegalic inclusion disease and fungus infection that occur during the first days of life [58] Nucleated red cells occur in the peripheral blood in a number of disorders but the occurrence of large numbers of normoblasts or reticulocytes usually indicates that the presence of hemolytic disease of some type is probable

The possibility of erythroblastosis particularly Rh disease should be suspected in pregnant women who give a history of a normal pregnancy followed by pregnancies that ended with jaundiced infants abortion or still birth. In about 40 to 50 per cent of the cases of ABO hemolytic disease the firstborn child is affected [304] The presence of marked edema or pallor at birth or the appearance of jaundice during the first 24 hours may be the first indication of the disease in the infant However all the abnormal clinical findings characteristic of the disease are sometimes absent at birth and for this reason the laboratory examinations are often of greater importance than the clinical features

Every effort is made to make an antenatal diagnosis of erythroblastosis by the use of appropriate laboratory procedures whenever possible All pregnant women should be typed to determine the ABO group and tested for the Rh factor routinely if the prospective mother is Rh negative the father should be tested for Rh factor and the mother should be tested for possible Rh sensitization at seven months and within two or three weeks of delivery [60] If anti Rh antibodies are not detected at that time erythroblastosis is unlikely if antibodies are present regardless of the titer the physician is alerted for the possibility of the disease A positive Coombs (antiglobulin) test on the fetal red cells (cord blood or infant s blood) definitely establishes the diagnosis If the husband is Rh positive the wife should be checked carefully for sensitization in each pregnancy because a normal course in preceding pregnancies is no guarantee that iso immune disease will not develop in subsequent pregnancies

Agglutinogens A and B were formerly considered to be responsible for from 5 to 9 per cent of the cases of erythroblastosis [59] [98] but more recent studies place the incidence at about 66 per cent [80] Erythroblastosis due to ABO incompatibility is usually much milder than that due to Rh incompatibility ABO incompatibility between mother and fetus exists in 20 per cent of pregnancies but erythroblastosis fetalis occurs in only 5 per cent of these (one per cent of the total) A possible explanation for the lower incidence and milder nature of ABO disease as compared to anti Rh disease is that the agglutinogens A and B occur on other tissue cells of the fetus as well as on the erythrocytes In such a circumstance the other tissue cells may take up the antibodies and spare the erythrocytes Rh agglutinogen apparently is largely or exclusively confined to the erythrocytes and consequently in this situation the red cells alone suffer from the reaction with antibodies [50] [98] Erythroblastosis due to ABO incompatibility is rarely associated with severe anemia. Marked hepatomegaly or splenomegaly is unusual The most significant clinical feature is the occurrence of jaundice during the first 24 hours When erythroblastosis is suspected an antiglobulin (Coombs) test should

be done on the baby's blood and tests for AbO and Rh factors should be done on the blood of the mother and the baby The occurrence of A or B incompatibility between mother and child accompanied by reticulocytosis and elevated serum bilirubin in the child is strong presumptive evidence of erythroblastosis[280] The indirect antiglobulin (Coombs) test performed on the infant's serum is nearly always positive in disease of this type[304]

TREATMENT

Infants with erythroblastosis should be treated in a hospital that is properly equipped and staffed to follow the course of the disease and carry out exchange transfusion at any time of day or night Once the diagnosis of hemolytic disease of the newborn has been established the treatment will depend on the severity of the disease Mild forms of the disease may need no treatment other than careful observation Therapy is directed toward preventing kernicterus that is due to the toxic action of the unconjugated bilirubin on cells in the brain. The indications for exchange transfusions in erythroblastosis fetalis listed by Allen and Diamond are [50]

1 Anemia hemoglobin of less than 13 0 gm per 100 ml on venous blood or less than 15 gm per 100 ml in capillary blood

2 Increased serum bilirubin (indirect reacting type) over 7 0 mg per 100 ml on cord blood a rapidly rising serum level exchange transfusion is performed as often as necessary to keep the serum level less than 20 mg per 100 ml

3 Prematurity except in the mildest cases

4 History of a previous baby with kernicterus

5 Reticulocyte count above 15 per cent

6 Maternal anti Rh titer of 1 64 or higher

Zuelzer and Cohen state that as a general rule exchange transfusion is performed if the serum bilirubin level in full term infants exceeds the following 10 mg per 100 ml in the first 24 hours 14 mg per 100 ml in the second 24 hours 17 mg in the third or fourth day[304]

The exchange transfusion performs several important functions In addition to lowering the level of serum bilirubin in the infant's blood it removes cells that are susceptible to hemolysis lowers the antibody content of the blood and supplies albumin that enables the infant's blood to bind a larger amount of bilirubin Repeated exchange transfusions may be required to keep the serum bilirubin level below 20 mg per 100 ml Following an exchange transfusion the infant is observed carefully and the serum bilirubin level is measured as often as is considered necessary

Fresh blood is used for transfusion whenever possible It is preferable to obtain the blood for transfusion prior to the birth of the infant In cross matching for transfusion the prospective donor cells should be tested against the mother's serum [80] [99] Prospective donors should be of the same Rh and ABO groups as the mother Rh negative blood of the mother's group is used if it is fairly certain that the disease is due to anti Rh agglutinins Group O blood is used when the destruction is thought to be due to anti A

or anti B agglutinins[99] ABO agglutinins in the donor blood incompatible with the babys cells should be of low titer and should be neutralized with A and/or B specific substances If no compatible donor can be found the mothers red cells separated from her plasma that contains the antibodies may be used[80] When the mothers blood is not available for cross matching it is probably best to select a group O Rh negative donor to cross match with the babys blood

If edema of congestive heart failure secondary to anemia is present phlebotomy is often necessary in order to lower the venous pressure The oxygen carrying capacity of the blood can be increased by means of trans fusion with packed red cells which only partially replaces the blood volume

Induction of labor at 37 weeks or later has been recommended in an effort to prevent stillbirth[280] Before advising this course the dangers of pre maturity must be considered In one study the fetal mortality rate was higher in the group with induced labor than in the group with spontaneous labor[300]

PROGNOSIS

The high mortality rate of erythroblastosis fetalis has been reduced by modern treatment and may be about 5 per cent[80] Proper treatment of heart failure in the infant and judicious induction of labor have improved the prognosis in this disease The most serious complication kernicterus is largely preventable by the use of exchange transfusions that keep the serum bilirubin level lower than 20 mg per 100 ml[81-83, 98]

The factors entering into the prognosis for future babies have been reviewed[80] The prognosis for subsequent babies after an instance of erythroblastosis that is due to ABO incompatibility is excellent because still birth is rare and kernicterus can usually be prevented In Rh incompati bilities if the husband is heterozygous 50 per cent of the offspring will be Rh negative and compatible with the mother If the husband is homozygous all babies will be Rh positive and the prognosis depends a great deal on the titer of the antibodies in the mothers blood If there is no history of previous stillbirth the prognosis even in heavily immunized mothers is generally good if the baby can be treated promptly

ILLUSTRATIVE CASE

The patient was the product of the seventh pregnancy of a 38 year old white female The mother was type A Rh negative and father was type A Rh positive There had been two previous neonatal deaths due to erythro blastosis Saline antibodies were positive in 1 4 dilution and indirect Coombs test was positive in dilution of 1 64 Pregnancy was uneventful labor lasted 6 hours and 21 minutes and terminated in a normal spontaneous delivery

The baby appeared to be in good condition in the delivery room He was well developed and weighed 2700 gm The skin was grayish in color and was covered with multiple small ecchymoses The umbilical cord was

icteric The baby was active when stimulated and cried vigorously The spleen edge extended 5 cm over the left costal margin and the liver 7 cm below the right costal margin

Laboratory Examination and Treatment Initial blood examination total bilirubin 4 mg hct 25 per cent hb 7 0 gm per 100 ml wbc 13 000 per cu mm nucleated red cells 150 per 100 leukocytes

A diagnosis of erythroblastosis was made and an exchange transfusion was performed After exchange transfusion the serum bilirubin level was 2 4 mg per 100 ml and the hematocrit 29 per cent The leukocyte count was 4800 per cu mm Five hours later another exchange transfusion was performed at a time when the serum bilirubin had risen to 6 mg per 100 ml and the nucleated red cell count had reached 337 per 100 leukocytes After the second exchange transfusion serum bilirubin level was 2 7 mg per 100 ml and the hematocrit was 36 per cent No further exchange transfusions were necessary and the patient was discharged on the thirteenth day at which time he was eating well and appeared healthy At this time the hematocrit was 46 per cent the white count was 12,000 per cu mm and the blood smear contained 5 nucleated red cells per 100 white cells

Comment This patient illustrates many of the common features of erythroblastosis The father was Rh positive the mother was Rh negative Two siblings had died previously in the neonatal period because of erythroblastosis The disease was anticipated in this patient At birth pallor ecchymoses splenomegaly and hepatomegaly were present the antiglobulin test was positive Because of the previous history in the family and the severe anemia exchange transfusion was performed even though the serum bilirubin level was only slightly elevated at the time of birth The necessity of the second exchange is debatable Although the level of the serum bilirubin level was not greatly elevated exchange transfusion was considered to be the safest course mainly because of the rate of increase in the bilirubin level The patient made an uneventful recovery

ACQUIRED HEMOLYTIC ANEMIA

Synonyms Auto immune Hemolytic Disease Acquired Hemolytic Icterus, Subacute or Chronic Idiopathic Hemolytic Anemia Lederer s Anemia

HISTORY

The history of "acquired" hemolytic anemia has been reviewed by Dameshek and Schwartz.[311] The first recognizable description of this type of

anemia has been credited to Hayem[310, 313] The "non congenital type of hemolytic icterus which appeared either during the course of other illnesses or without known cause was also described by Widal Abrami and Brule in 1908[3, 3-3] The name acquired" hemolytic icterus was given to these cases The occurrence of reticulocytosis and auto agglutination the absence of apparent hereditary influences and the absence of significant abnormalities in the hypotonic saline test were noted As a result of these observations the "acquired type of hemolytic icterus of Hayem and Widal was distinguished from the congenital type of Chauffard and Minkowski Acute subacute and chronic cases were recognized and hemolysins were sometimes demonstrated in the acute cases Lederer s anemia" an acute hemolytic anemia of short duration[317] appears to be an example of the acute type of this disorder rather than a separate disease[310, 311]

The redemonstration of spherocytes and hemolysins in acute cases of the acquired type by Dameshek and Schwartz in 1940[311] and the discovery of the antiglobulin or Coombs test in 1945 by Coombs Mourant, and Race[307] were followed by a marked increase in interest in this syndrome This has led to numerous studies on the demonstration production action and nature of auto antibodies" Most of these studies have been reviewed by Dacie[310] At the present time this type of anemia is often spoken of as auto immune hemolytic disease"

PATHOGENESIS

Neither the cause nor the mechanism of hemolysis in this disorder is known but several possibilities have been considered The occurrence of abnormal agglutination tests such as the Coombs antiglobulin test has been interpreted as an indication that auto antibodies are produced as a response to an antigen that consists of the patient s own cells or parts of cells that have been altered in some way While it appears possible that drugs viruses or metabolic products might alter an individual s erythrocytes sufficiently to make them antigenic and lead to the production of auto antibodies" the action of these antibodies against normal erythrocytes as well as those of the patient makes this thesis appear somewhat doubtful[310] Another possibility is that the auto antibody reaction is an incidental property of a globulin produced by the reticuloendothelial system that is involved in some disease such as lymphosarcoma or infection[30, 309] The abnormal proliferation and destruction of the cells in the protein forming tissue might conceivably lead to the production of protein that is abnormal qualitatively as well as quantitatively The report that chromic and ferric ions in the presence of globulin or serum whether autologous homologous or heterologous could bind globulin to the erythrocyte and produce a positive antiglobulin (Coombs) test appears to support the second possibility[314] The balance of the evidence at the present time appears to be against the concept that the antibodies are produced in response to an antigen derived from the patient s own cells but the possibility that antibodies of low specificity are formed in

response to mutant erythrocytes or against abnormal or damaged lympho cytes or against material derived from both lymphocytes and erythrocytes cannot be dismissed entirely[318]

Although the mechanism of the actual destruction of the erythrocytes in vivo is not entirely understood the ability of antibodies to cause auto agglutination spherocytosis and erythrophagocytosis appears to be im portant[319] Auto agglutination may play a role by causing stasis of blood in the spleen that leads to destruction of the erythrocytes by lysis or phagocytosis[306, 3 0] It has been established that the spleen liver lymph nodes bone marrow and lungs are the organs that are most concerned in the actual destruction of the erythrocytes[319, 314] The results of splenectomy in this disorder probably depend on the relative importance of two functions of the spleen which may act as an organ of cellular destruction and as a site of antibody production

CLINICAL MANIFESTATIONS

Auto immune hemolytic anemia may arise in association with some other disease when it is termed secondary" or "symptomatic" It may also occur when no other disease is demonstrable in which circumstance it is classified as "primary" or "idiopathic In one group of 57 patients 34 were classified as idiopathic and 23 were classified as symptomatic 16 of the 23 symptomatic cases had some form of malignant disease[3 8] In a series of 49 patients most of whom were ambulatory 39 were of the idiopathic type and 10 were secondary 7 of the 10 secondary cases followed virus pneumonia[310] In another report of 166 patients the anemia was considered to be "secondary" to other diseases in 85[3 0] The symptomatic or secondary cases have oc curred most often in association with primary atypical pneumonia chronic lymphocytic leukemia, lymphosarcoma Hodgkin s disease disseminated lupus and infectious mononucleosis[3 8, 309, 319] Less common associated dis eases are sarcoidosis tuberculosis[319] Felty s syndrome[308] and carcinoma[31]

The idiopathic disorder occurs at any age in either sex but females are affected more often than males[309, 319] The severity and duration of the dis ease may vary greatly At times the onset of the disease is sudden and the course is rapid and fulminating In such patients the course is characterized by high temperature rapidly progressing weakness and dyspnea Anemia becomes more severe icterus is usually apparent and hemoglobinuria some times occurs It is in this type of patient that hemolysins are more likely to be demonstrated More often the course of the disorder is a chronic one that persists with varying degrees of severity over a period of months or years Spontaneous remissions sometimes occur[3 4] Exacerbations of the disease are said to occur more often in the winter months[308] When the disorder is asso ciated with the cold type of auto antibodies Raynaud s phenomena and hemoglobinuria may appear on exposure to cold[309] A subacute form that is intermediate in severity between the acute and chronic form also occurs

In symptomatic auto-immune hemolytic disease the clinical features are

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PATHOGENESIS

Neither the cause nor the mechanism of hemolysis in this disorder is known, but several possibilities have been considered. The occurrence of abnormal agglutination tests, such as the Coombs antiglobulin test, has been interpreted as an indication that "auto antibodies" are produced as a response to an antigen that consists of the patient's own cells or parts of cells that have been altered in some way. While it appears possible that drugs, viruses, or metabolic products might alter an individual's erythrocytes sufficiently to make them antigenic and lead to the production of "auto antibodies," the action of these antibodies against normal erythrocytes, as well as those of the patient, makes this thesis appear somewhat doubtful.[310] Another possibility is that the "auto antibody" reaction is an incidental property of a globulin produced by the reticuloendothelial system that is involved in some disease, such as lymphosarcoma or infection.[31, 309] The abnormal proliferation and destruction of the cells in the protein forming tissue might conceivably lead to the production of protein that is abnormal qualitatively as well as quantitatively. The report that chromic and ferric ions in the presence of globulin or serum, whether autologous, homologous, or heterologous, could bind globulin to the erythrocyte and produce a positive antiglobulin (Coombs) test appears to support the second possibility.[314] The balance of the evidence at the present time appears to be against the concept that the antibodies are produced in response to an antigen derived from the patient's own cells, but the possibility that antibodies of low specificity are formed in

inadequately diluted serum because of the prozone phenomenon from the use of insufficiently washed red cells[3,4] and probably from other causes The Coombs test may be positive for as long as one year before the anemia becomes evident in patients with lymphosarcoma[318] The indirect antiglobulin test is usually positive during active hemolysis and usually negative during clinical remissions[3,9] It has been recommended that tests for free auto antibodies as well as the Coombs test be carried out, particularly on patients who have received corticosteroids[312] Cold agglutinins are usually demonstrated without difficulty if they are responsible for the anemia since the titer is usually high in this circumstance[310] The clinical significance of the cold agglutinins may be related more to their ability to agglutinate or sensitize the patient s erythrocytes at relatively higher temperatures (37° C) than to the height of the agglutinin titer at low temperatures (2° C)[310]

DIAGNOSIS

The diagnosis of auto immune hemolytic anemia must be considered in previously well patients who present because of anemia and also in patients who develop anemia while they are under treatment for some previously diagnosed primary disease

The anemia is first identified as the hemolytic type by the appropriate tests such as the reticulocyte count the serum bilirubin determination the bone marrow examination and the measurement of the urobilinogen excretion Additional more specific tests such as the antiglobulin (Coombs) test hemoglobin electrophoresis and the osmotic fragility test are performed in order to categorize the anemia more accurately If the Coombs test is positive the diagnosis of auto immune hemolytic disease can be considered as virtually established if this test is negative and the other special procedures do not indicate any other diagnosis auto immune hemolytic disease is not excluded In either circumstance if it is decided that the patient has auto immune hemolytic disease a thorough investigation is made for some primary disease such as malignant lymphoma carcinoma and the other acute and chronic disorders known to be sometimes associated with this type of anemia If no primary disease is demonstrated, a tentative diagnosis of primary or idiopathic auto immune hemolytic disease is made with the realization that some underlying disease may be present but may remain unrecognized for months and even years Because of this possibility periodic follow up examinations are desirable

When auto immune hemolytic anemia appears in patients who are under treatment for some known disease the onset may be abrupt almost crisis like or insidious When the hemolytic process is severe the diagnosis is usually established by the evidence of hyperhemolysis associated with a positive antiglobulin test When the process is less severe and the evidences of hemolysis and blood regeneration are not so apparent the presence of a hemolytic process may not be suspected If the antiglobulin test is negative it may be difficult to determine whether the anemia is the result of auto

those of the underlying disorder plus those associated with the hemolytic anemia. At times the hemolytic element may be so severe that it dominates the clinical picture and at other times it may be merely an incidental finding. When hemolytic anemia follows virus pneumonia, the anemia, which may be severe, usually appears suddenly at the end of the second or third week of illness and disappears spontaneously in one or two weeks.[318] In diseases such as lymphosarcoma the hemolytic anemia may precede other evidence of the primary disease by months, or it may not appear until after the underlying disease has been under observation and treatment for several years.[318] The development of auto-immune hemolytic disease may be the first manifestation of a recurrence of carcinoma of the bowel.[316]

The main abnormalities on physical examination in the idiopathic cases are pallor and splenomegaly. In addition, patients with the symptomatic type may manifest evidence of the underlying disease, such as lymphadenopathy, hepatomegaly, or evidence of disease in the skin, lungs, and gastrointestinal tract.

LABORATORY EXAMINATION

Any degree of anemia may occur in either the idiopathic or symptomatic forms of disease. In one series of 57 cases the hemoglobin varied from 2.5 gm. to 12.5 gm. per 100 ml.[3] The anemia is usually macrocytic (MCV $>$ 94 cu. microns) but a stained smear of the peripheral blood often reveals many microcytes, polychromatophilia, poikilocytosis, and the presence of normoblasts. Spherocytosis is often present during periods of crisis and is usually absent during quiescence.[3,4] Reticulocytosis is usually present, but reticulocytopenia occurs in some patients.[308] Leukocytosis is common during periods of active hemolysis and the count may exceed 30 000 per cu. mm.; the increase is mainly in the granulocytic series and myelocytes may be found in the peripheral blood.[3,9] Neutropenia sometimes occurs in chronic cases[3,9] but is unusual during episodes of crisis.[308] Erythrophagocytosis by monocytes is sometimes evident on plain smears or on smears made from the buffy coat and may suggest the diagnosis.[3,8,309] The platelet count is usually normal or moderately reduced but severe thrombocytopenia is found in some patients.

The other evidences of a hemolytic process such as increased plasma bilirubin, increased fecal urobilinogen excretion, and normoblastic erythroid hyperplasia of the bone marrow can usually be demonstrated. Osmotic fragility and mechanical fragility are irregularly increased during crisis but are usually normal during periods of quiescence.[3,4] Hemoglobin electrophoresis reveals the normal A type of hemoglobin.

One of the most important examinations is the direct antiglobulin or Coombs test.[307] In some laboratories it is positive with but very rare exceptions in auto-immune hemolytic anemia of both the warm and cold antibody types.[309] However, the percentage of positive tests on patients who appear to have this syndrome varies greatly from one laboratory to another. False negative tests may result from the use of a weak serum, from the use of

nosis could be established Accordingly the patient was given prednisone 20 mg every 8 hours beginning on June 24 On June 26 this was reduced to 10 mg four times a day and at discharge on July 3 the dose was reduced to 5 mg three times a day There was a striking improvement in her blood count and on September 16 her hematocrit was 42 per cent and the reticulocyte count had fallen to 1 5 per cent On November 4 one month after her prednisone dosage had been reduced to 2 5 mg three times a week the patient was asymptomatic and the hematocrit was 39 per cent

Comment This patient had evidence of a hemolytic anemia and the positive direct Coombs test indicated that the anemia was of the extra corpuscular or auto immune type No primary diagnosis was established and the case might be considered to be "idiopathic" However various features strongly suggest the presence of some underlying disease and in this circumstance the patient must be examined periodically for additional evidence of some primary disorder The anemia has responded well to treatment with prednisone for two years although the Coombs test remains positive

HEMOGLOBINURIA

Hemoglobinuria is an abnormal clinical sign that is found in a number of different diseases Excellent reviews have been published by Ross[336] and Ham[13] Ham has classified the clinical conditions in which hemoglobinuria occurs as follows

1 strenuous exercise such as running from two to twenty six miles
2 march hemoglobinuria where a lordotic posture appears to be of some importance
3 infusion of hypotonic solutions by intravenous injection or in performing transurethral resection
4 intravascular hemolysis in transfusion reactions and severe erythroblastosis fetalis
5 thermal injury
6 injury from chemicals such as arsine (arsenuretted hydrogen)
7 action of hemolytic drugs such as acetylphenylhydrazine beta naphthol and sulfonamides
8 favism
9 infested red cells (malaria Oroya fever)
10 paroxysmal cold hemoglobinuria
11 paroxysmal nocturnal hemoglobinuria

The prognosis of patients with the symptomatic type of anemia is mainly that of the underlying disease. The prognosis is generally favorable when the disorder follows a virus infection and is poor when it is associated with malignancy or one of the collagen diseases. In the idiopathic types spontaneous recovery is rare except in acute cases of the "Lederer" type; in the *chronic cases complete recovery* rarely occurs but the prognosis is better than in patients with malignant disease. The occurrence of severe anemia, reticulocytopenia, thrombocytopenia and leukopenia appears to be associated with a poor prognosis.[298] The commonest causes of death in one group of 18 patients with idiopathic auto-immune hemolytic disease were cardiac failure caused by anemia, acute renal failure and septicemia.[308] Cardiac failure, infections and hemorrhages were the commonest causes of 14 deaths in the symptomatic cases.

ILLUSTRATIVE CASE

This 53 year old white housewife had been under observation for five years as a tuberculous suspect but the diagnosis was never established. On a periodic chest roentgenogram it was noted that hilar adenopathy had developed and the patient was admitted to the hospital for investigation. The patient admitted vague chest pains and irregular cough on occasions. She denied weight loss, pruritus or other symptoms.

Physical Examination. The posterior cervical and axillary lymph nodes were enlarged and measured 0.5 to 1 cm in diameter. The spleen was felt two fingerbreadths below the left costal margin. Physical examination was otherwise within normal limits.

Laboratory Examination. Admission blood studies: hb 7.1 gm per cent; hematocrit 27 per cent; rbc 1.9 mil per cu mm; retics 22.3 per cent; wbc 18,000 per cu mm. Differential count (per cent): bands 4, segmented 50, lymphocytes 40, monocytes 1, eosinophils 4, basophils 1. The serum protein was 8.9 gm per 100 ml; albumin 3.5 gm per 100 ml; globulin 5.4 gm per 100 ml. The direct antiglobulin (Coombs) test was positive and the Wassermann test was anticomplementary. Blood cultures, tuberculin skin test, platelet counts and examination for L.E. cells were normal. Plasma electrophoresis indicated increased total protein, increased gamma globulin, decreased albumin and beta globulin with two peaks; the pattern was considered suggestive of Boeck's sarcoid. Bone marrow aspiration revealed marked normoblastic hyperplasia; plasma cells and eosinophils appeared to be slightly increased.

Regular roentgenograms and laminograms of the chest suggested a slight enlargement in the right hilus area. On bronchoscopy no mass was visualized. Biopsies of the right prescalene node and right axillary node were interpreted as "hyperplastic lymphadenitis."

Treatment and Course. The patient was thought to have a hemolytic anemia of the auto-immune type. Although sarcoid, lymphosarcoma and disseminated lupus were suspected as underlying primary diseases, no diag

test can be demonstrated during an attack. More specific tests establish the diagnosis. In the Rosenbach test an attack is reproduced by immersing the hands or feet in ice water for ten to twenty minutes.[335, 337] In various modifications of the Donath-Landsteiner test hemolysis is demonstrated after the blood has been chilled in vitro.[337]

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

Paroxysmal nocturnal hemoglobinuria is a rare disorder of unknown cause that occurs in both sexes.[6, 3, 7, 33, 336] Although the disease has been reported in patients 5½ and 52 years of age, it usually appears during the third or fourth decade.[336] The disease is characterized by chronicity, spontaneous remissions and exacerbations, and refractoriness to therapy. The factors that are concerned in the excessive hemolysis found in this disease—the defect in the erythrocyte, the essential plasma factors, and the influence of pH—are discussed in Chapter III. It has been reported recently that a marked reduction of the acetylcholinesterase activity in the erythrocytes is a consistent finding in this disease.[3, 5] This defect is located in the stroma of the cell.

Macrocytic hemolytic anemia of moderate or severe degree occurs. The erythrocyte count is usually from 2.0 to 3.0 mil. per cu. mm. Leukopenia and thrombocytopenia of moderate degree are characteristic. The usual evidences of hemolytic anemia, such as reticulocytosis (20 or 30 per cent), elevated level serum bilirubin (5 to 6 mg. per 100 ml.), and normoblastic hyperplasia of the bone marrow are found.[336] The osmotic fragility and the antiglobulin (Coombs) tests are normal. Hemosiderinuria and increased amounts of plasma hemoglobin are found constantly; hemoglobinuria is absent if the renal threshold is not exceeded.[336] The diagnosis is established by demonstrating the erythrocyte abnormality in the acid serum test devised by Ham,[33, 334] or by the hemolysis test that utilizes thrombin devised by Crosby.[3, 9] Another test is performed by adding 10 per cent by volume N/5 HCl and 10 per cent by volume of a 50 per cent suspension of the patient's washed corpuscles to serum obtained from blood that was defibrinated in an open flask; if the test is positive, frank hemolysis is present.[337]

The course of the disease is a chronic one that continues for many years in some patients. In the fatal cases death is caused not only by anemia and infections during acute episodes but also by thromboses of the cerebral, coronary, portal, hepatic, and pulmonary vessels. When severe anemia is a problem, transfusion of red cells rather than whole blood is recommended because the administration of plasma increases the likelihood of a hemolytic reaction and increased destruction of the patient's erythrocytes.[3, 7, 33] Splenectomy, the administration of alkali, and the adrenocorticosteroids do not produce significant benefit.[337] The value of anticoagulants in preventing thrombosis has not been established.[33]

Detectable degrees of hemoglobinemia are found in normal persons but the level does not exceed 5 mg per 100 ml and is usually in the range of 2 or 3 mg per 100 ml of plasma [3,8,330] Increased amounts usually from 20 to 60 mg per 100 ml were found in a number of patients with acquired hemolytic anemia thalassemia major and sickle cell anemia [3,8] Hemosiderinuria was found in every patient with hemoglobinemia and in general the amount of iron staining pigment varied with the concentration of the plasma hemoglobin The normal renal threshold for hemoglobin is reported to be around 90 to 130 mg per 100 ml [3,7,8,9,331,33] Hemoglobinuria occurs only after rather severe intravascular hemolysis and is rarely seen in sickle cell anemia, thalassemia or acquired hemolytic anemia [3,8,33] Although the conditions are rare hemoglobinuria occurs frequently in transfusion reactions favism blackwater fever and in the paroxysmal hemoglobinurias

PAROXYSMAL COLD HEMOGLOBINURIA

Paroxysmal cold hemoglobinuria is a rare disorder The sudden attacks of intravascular hemolysis and hemoglobinuria that occur on exposure to cold are due to the presence of an autohemolysin in the serum The mechanism of the production of the attacks is discussed in Chapter III The Wassermann reaction is reported to be positive in over 90 per cent of patients with the disorder and unquestionable clinical evidence of syphilis is found in about 30 per cent [335]

The attacks are precipitated by exposure to cold but there is a wide range of variability in the amount of chilling that is necessary no more than the opening of a door from an unheated room may be required [33] The interval between the chilling and the onset of symptoms may vary from a few minutes to seven or eight hours The prodromal period is apt to be associated with malaise headache abdominal cramps and pains in the back and legs The actual paroxysm is usually manifested by a shaking chill followed by a temperature rise of 102 or 104° F that lasts for several hours After the chill the voided urine usually appears dark red or brown urine of this character may be passed in only the first or first few specimens after the chill or over a period of two or three days During the episode mild jaundice splenomegaly and hepatomegaly are often demonstrable [33,336] Severe leukopenia that is the result of the virtual disappearance of the granulocytes from the peripheral blood also occurs [336]

Treatment during an attack consists of keeping the patient in a warm environment and administering transfusions when necessary Between episodes exposure to cold is avoided and any underlying disease is treated Many patients remain asymptomatic during these intervals [33]

The diagnosis is made by the history and by abnormalities that can be demonstrated in the laboratory tests The degree of the anemia and the evidence of blood regeneration depend on the severity of the hemolysis Hemoglobinemia hemoglobinuria and a positive antiglobulin (Coombs)

spleen was the major site of destruction. A correlation was found between the rate of red cell destruction and the degree of anemia. The presence of hemolytic anemia in liver disease was also indicated by the animal experiments of Higgins and Stasney.[347] They exposed rats to carbon tetrachloride by inhalation for short periods each day for twelve weeks. At the end of this time the rats developed "typical" atrophic cirrhosis of the liver and macrocytic anemia. In addition there was associated evidence of increased hemolysis in the form of icterus, increased hemosiderin in the organs, erythroid hyperplasia in the bone marrow, and an increase in reticulocytes.

The demonstration of the hemolytic nature of the anemia in cirrhosis of the liver affords the best explanation of the anemia in the majority of patients who have a macrocytic anemia associated with a normoblastic marrow that does not respond to treatment with folic acid, vitamin B_{12}, or liver extract. Factors other than hyperhemolysis are important in the small percentage of patients with the megaloblastic type of bone marrow who have responded to specific treatment.[341, 343, 344] The anemia in most of these patients has been more severe than that usually seen in cirrhosis. The erythrocyte count has been in the neighborhood of 1.0 mil. per cu. mm. in most patients and less than 2.0 mil. per cu. mm. in nearly all; the mean corpuscular volume usually has been over 130 cu. microns. In such patients hematologic recovery has followed the administration of folic acid,[341] vitamin B_{12},[344] liver extract,[339, 344] and ascorbic acid.[338] The possibility of a coexisting pernicious anemia was excluded by gastric analysis[344] or determinations of the serum vitamin B_{12} level.[338, 341] It appeared that deficient intake of folic acid, vitamin B_{12}, or ascorbic acid was an important factor in the defective erythropoiesis in these patients because they gave histories of grossly deficient diets for long periods. The recognition of at least two types of macrocytic anemia in cirrhosis of the liver probably explains the conflicting reports of the presence[345] and absence[339] of the antipernicious anemia factor in livers of patients who died with cirrhosis. Still another possibility to be considered is that alcohol may directly depress marrow activity. This was suggested by the observation that a reticulocytosis occurred in patients with cirrhosis even though they were kept on a diet deficient in erythropoietic factors if the ingestion of alcohol was prevented.[341]

HYPERSPLENISM

Hypersplenism is the term used to denote a syndrome characterized by the following: (1) splenomegaly, (2) anemia, leukopenia, thrombocytopenia singly or in combination, (3) a marrow that has normal or increased cellularity, (4) correction of the blood picture by splenectomy.[349, 350]

ANEMIA ASSOCIATED WITH CHRONIC LIVER DISEASE

Anemia occurs often in patients with chronic liver disease and sometimes undergoes spontaneous remission.[341, 347] In one series of 132 patients with different types of liver disease, anemia was found in 78 per cent.[347] The incidence is even higher in patients with atrophic cirrhosis.[341, 34, 34, 347] The degree of anemia, which is rarely severe, cannot be correlated with the duration of the liver disease or with the clinical outcome.[341, 347] The morphologic characteristics of the anemia, which may be macrocytic, normocytic or hypochromic microcytic, depend on what mechanisms are responsible for the anemia. Either blood loss, increased hemolysis or deficient erythropoiesis may be the most important factor in a particular individual.

The hypochromic microcytic type of anemia was found in only 12 per cent of one series.[34] Its occurrence indicates iron deficiency, which is usually the result of chronic blood loss from the gastrointestinal tract. Iron deficiency may occur as the result of repeated severe hemorrhages from ruptured esophageal varices or mild chronic blood loss from irritation of the gastrointestinal tract. Increased pressure in the portal circulation and a defect in blood coagulation may be contributing factors.

In chronic liver disease the anemia is usually macrocytic but is sometimes normocytic.[341, 34, 347] The mean corpuscular volume has been found to range from 95 to 160 cu microns but is usually between 100 and 120.[341, 34] The marked anisocytosis and poikilocytosis which are usually seen in pernicious anemia are absent in chronic liver disease, and nucleated red cells rarely appear in the peripheral circulation. Erythropoiesis is usually normoblastic in type.[341] The reticulocytes are often slightly increased and the leukocyte and platelet counts are normal or slightly reduced.

The mechanism responsible for the macrocytic anemia of cirrhosis of the liver has been clarified by the studies of Jandl.[341] Sixteen of 20 patients with cirrhosis and anemia who were studied were found *to have a definite hemolytic anemia*, evidenced by the triad of elevated excretion of fecal urobilinogen, persistent reticulocytosis and increased concentration of the erythrocyte precursors in the bone marrow. The extracorpuscular nature of the hemolytic process was shown by the survival times of transfused normal cells that were determined by the Ashby differential agglutination method. The exponential type of survival curve that was found suggested a random destruction of the red cells in a local site, such as the liver or spleen. Additional studies that utilized erythrocytes tagged with Cr^{51} indicated that the

tinues even though a partial hematologic relapse not infrequently develops some months after splenectomy

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This syndrome occurs in a variety of disorders that are associated with splenomegaly. Among these are congestive splenomegaly associated with portal hypertension of either the intrahepatic or extrahepatic variety; chronic infections such as tuberculosis, kala-azar, malaria, subacute bacterial endocarditis, syphilis, brucellosis, fungus diseases; diseases of the lymphoma group, particularly lymphosarcoma, giant follicle lymphoma and Hodgkin's disease; diseases of lipid metabolism (Gaucher's disease); diseases of uncertain etiology such as rheumatoid arthritis, sarcoidosis and collagen diseases, particularly disseminated lupus erythematosus; splenomegaly of undetermined origin. Usually hypersplenism is simply one aspect of an underlying disease that is not confined to the spleen. In the vast majority of patients the diagnosis is established before splenectomy or at the time of operation. However, in a small percentage of patients the cause of the splenomegaly remains obscure even after histologic study of the spleen and other biopsy material removed at operation. Such cases are termed "idiopathic" or "primary."[349, 351]

Various mechanisms have been suggested to explain the changes that occur in the peripheral blood in hypersplenism. Doan and his group, largely as the result of supravital staining of splenic material, have considered that sequestration and destruction of the cellular elements by the abnormal spleen are the important factors.[350] A different concept is offered by Dameshek, namely that the enlarged spleen exerts an inhibiting effect on the bone marrow by means of an unidentified humoral factor.[349] Others consider that leukocyte antibodies are important in many splenic neutropenia syndromes,[350] although diseases characterized by altered antibody production, such as idiopathic thrombocytopenic purpura and auto-immune acquired hemolytic anemia, are excluded from the "hypersplenic" group by some.[349] At the present time the mechanism involved in "hypersplenism" cannot be considered as definitely established and it is not certain that the same mechanism is the responsible one in every patient with the syndrome.

The decision regarding the advisability of splenectomy can be made only after careful consideration of the individual patient. When the presence of hypersplenism is suggested because of the occurrence of splenomegaly, cytopenia and an apparently active cellular marrow, efforts are made to establish the identity of the underlying disease. Splenectomy relieves only one aspect of the underlying disorder and rarely if ever effects a cure of the primary disease. If the underlying disease is amenable to more definitive treatment, splenectomy may not be necessary because the hypersplenism syndrome may disappear after such therapy. When the nature and course of the underlying disease are such that an early fatal outcome is expected, splenectomy is rarely a justifiable procedure. If the nature of the underlying process cannot be diagnosed, or if it is identified as one for which no satisfactory therapy is available, splenectomy is a justifiable form of treatment if the presence of anemia, neutropenia, or thrombocytopenia is disabling or hazardous to the patient. In such circumstances gratifying results occur often but not invariably. Clinical improvement in the patient often con

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POLYCYTHEMIA VERA

Synonyms Primary Polycythemia Vaquez's Disease, Osler's Disease Polycythemia Rubra, Erythremia

Historical Vaquez in 1892 described the first instance of polycythemia that was not associated with pulmonary or cardiac disease[47] The second description of such a patient was published by Cabot in 1899[9] In 1903 Osler collected 8 cases 4 from the literature and 4 of his own and pointed out many features of the disease[4] In 1908 he clearly defined the disorder and discussed the course and prognosis as well as the therapeutic use of bleeding oxygen and irradiation of the spleen[48] Excellent comprehensive reviews of the disease have been written by Harrop[6] Lawrence Berlin, and Huff[38] and Pike[50]

PATHOGENESIS

The etiology of polycythemia vera is unknown Because of the prolifera tion of all the cell lines in the bone marrow and the frequent transformation of polycythemia vera into leukemia myelofibrosis and other related syn dromes polycythemia vera has been considered to be a myeloproliferative disorder[9 14 3 41 6] According to this concept the cytoplasmic reticulum retains its potential for development into various cell lines and responds in different ways to unknown stimuli The clinical course of the disease seems to be best explained by this hypothesis at the present time

Because of the known development of polycythemia in response to anoxia numerous attempts have been made to explain polycythemia vera by a nar row anoxia mechanism of some sort but none has been satisfactory[3 55] The greater frequency of leukocytosis and thrombocytosis as well as the finding of normal arterial oxygen saturation in polycythemia vera serve to differen tiate it from anoxic polycythemia[9]

The increased level of erythrocytes in polycythemia vera is due to in creased production of cells rather than to an increased life span of the cells Studies with radioactive iron showed that the plasma iron turnover in this disease is five times normal[3] Studies on the life span of the cells have shown that it is normal[4] or that there is a dual population of cells with a normal span together with cells that have a very short life span[4] The stimulus for the increased erythropoiesis in this disease has not been identified but ex tracts of plasma from patients with polycythemia vera have been reported to stimulate erythropoiesis when injected into test animals[10 41]

CHAPTER V

Polycythemia

AN INCREASE in red blood cells above the normal number may be *relative* or *absolute* If the volume of fluid in which the red blood cells are suspended is reduced an increase in their concentration results The number of cells per unit volume of whole blood is increased and this increase is reflected in the red blood cell count and the hematocrit. The total red cell mass remains unchanged Such a condition of *relative* polycythemia exists in any situation where the plasma volume is reduced without a concomitant reduction in red cell mass This relative polycythemia is most frequently associated with a shift or loss of body water and in such circumstances the red cell count and hematocrit determination are valuable aids in the recognition and quantification of such changes In interpreting the hematocrit it must be remembered that osmotic changes associated with alterations of fluid volume may alter the volume of the red blood cell Ingress or egress of water from the red cell in response to altered osmotic forces outside the cell may be sufficient to affect the hematocrit (Fig 20) When this occurs the red blood cell count more accurately reflects the loss of plasma volume

An *absolute* increase in red blood cell mass may occur either as the result of a physiologic response to tissue anoxia or in the pathologic state known as polycythemia vera. In the first instance the polycythemia is secondary to some recognized inciting cause while in the second the polycythemia is classified as "primary" since no inciting cause has been identified [8]

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The most common clinical manifestations are referable to the cardiovascular system Complaints such as dyspnea on exertion ankle edema angina pectoris palpitation headache dizziness and intermittent claudication are present in about two thirds of the patients when first seen [49]

In about one third of the patients gastrointestinal symptoms such as epigastric pain nausea, bloating vomiting and constipation are prominent The incidence of duodenal ulcer demonstrable by x ray is reported to be 10 to 19 per cent [38 49 50] Both hematemesis and melena occur not infrequently and either may be the first manifestation of the disease On occasion the patient's history of severe bleeding may be doubted because of the failure of the laboratory tests to demonstrate the degree of anemia suggested by the history

Other symptoms occur much less often in polycythemia vera but are recognized as part of the disease In some patients severe pruritus and night sweats are troublesome and constitute the main complaint Patients are sometimes treated for conjunctivitis" for a time before the underlying disease is recognized. Other less common complaints are those due to gout and those related to the genitourinary system such as hematuria dysuria and frequency

On physical examination often the most striking feature is the appearance of the patient—a ruddy and florid face telangiectasia of the cheeks and nose and purplish cyanosis of the lips and ears The appearance frequently suggests the diagnosis but the countenance of the middle aged man who has spent most of his life out of doors and indulged freely in alcohol may be virtually indistinguishable Marked reddening is often evident in the palms conjunctivae pharynx and vaginal mucous membranes Examination of the fundus frequently reveals retinal veins that are distended and dark purple in color Hypertension is present in about half of the patients The spleen is enlarged in about three fourths of the patients it is usually firm and rarely extends as low as the umbilicus unless the disease has been present for some years [61] Late in the course of the disease especially when leukemia or myelofibrosis has developed the spleen may become greatly enlarged and fill most of the abdominal cavity Hepatomegaly occurs in about one third to one half of the patients

LABORATORY FINDINGS

The laboratory data in patients with polycythemia vera illustrate the involvement of all the marrow elements and not the red cells alone [1 48 54] The untreated patient typically has an erythrocyte count in the range of 6 to 10 million a hemoglobin between 18 and 24 gm per 100 ml and a hematocrit that ranges from 55 to 80 per cent [9] The total red cell mass is increased, but the plasma volume may be normal, slightly elevated or even decreased.[1 5] The leukocyte count is usually elevated In our series of 50 patients a range of 5400 to 88 000 per cu.mm was found 70 per cent of patients had counts higher than 10 000 per cu mm [49] a figure comparable to

Most of the clinical manifestations of polycythemia vera can be explained by the alterations in the peripheral blood There is a definite increase in total blood volume to two or three times the normal value that is due almost entirely to an increase in the total mass of circulating red cells Associated with this increase in erythrocytes there is an increase in blood viscosity that may be five to eight times normal [6, 43] The increased blood volume and the slowed circulation in the congested capillaries are responsible for the plethoric appearance of the patient and the ruddy cyanosis which appears when the level of the non oxygenated hemoglobin in the capillaries rises above 5 gm per 100 ml [8] The increased blood volume and viscosity place an added load on the heart with the result that enlargement of the heart congestion of the pulmonary vessels and evidence of cardiac decompensation may develop These often improve markedly when the underlying disease is treated effectively Vascular disease is present in many patients with this disease The frequent occurrence of thrombosis in this disorder appears to be related to the presence of the vascular disease in association with the increased blood viscosity decreased blood flow and increased number of the blood platelets

One of the puzzling features of polycythemia a disease characterized by thrombosis is the frequent occurrence of abnormal bleeding The distention of the blood vessels has been considered a possible explanation [38] In addition abnormalities in the quality of the blood clot and in clot retraction have been demonstrated It has also been demonstrated that the presence of very large numbers of platelets interferes with the early stages of blood coagulation [34] Still another factor that may be important is the occurrence of increased fibrinolysis that has been demonstrated in polycythemia vera but not in secondary polycythemia [6]

CLINICAL MANIFESTATIONS

Polycythemia vera occurs oftener in males than in females the ratio being about 1 3 1 or 2 1 [16, 35, 38, 49, 6] The disease appears to be more common in Caucasians than in Negroes in a series of 50 patients with polycythemia vera seen at the University of Virginia Hospital the ratio of Caucasians to Negroes was 24 1 while in the total hospital admissions the ratio of Caucasians to Negroes was 4 5 1 [49] Clinical manifestations of the disease appear most often between the ages of 40 to 60 though the disease has been reported in children [16, 38] In the authors series the age at onset ranged from 21 to 68 years but only 10 per cent of the patients were below the age of 40 [49]

Most of the symptomatology of polycythemia vera is related to the increased blood volume the associated vascular disease and the tendency to hemorrhage and thrombosis

The onset is usually insidious and the manifestations variable Weakness easy fatigue irritability and dizziness are common early complaints At other times the onset is abrupt and the first indication of the presence of the disease is the occurrence of a catastrophe such as a gastrointestinal hemorrhage myocardial infarction or the development of hemiplegia

13 2 years in patients treated with radiophosphorus and 6 7 years in patients treated with various methods other than P[3] Survival times in individual patients vary from a few months to 25 years [18] The commonest causes of death are thrombotic episodes hemorrhage leukemia and other myelo proliferative states In one series 30 per cent died from thrombosis and 30 per cent from leukemia or some allied disorder [18] In Lawrence s series the causes of death in 57 patients treated with P[3] were as follows chronic mye logenous leukemia, 11 (6 were acute terminally) cerebral hemorrhage 7 coronary thrombosis 8 congestive heart failure 9 carcinoma 8 postopera tive deaths 5 miscellaneous 4 unknown 5 [38] In our own series of 24 pa tients who died 12 had received no form of irradiation therapy and 12 had received treatment with P[3] or x ray The causes of death in the 12 who had received no irradiation were heart failure or cerebrovascular accident 8 postoperative hemorrhage 1 carcinoma 1 miscellaneous (peritonitis mul tiple sclerosis renal insufficiency) 3 The cause of death in 12 who had received P[3] or x ray were heart failure or thromboses 3 myelogenous leukemia (acute) 3 myeloid metaplasia 2 miscellaneous (hepatic cirrhosis unknown) 3 [49]

Although the complications of thrombosis and hemorrhage are the most common serious developments in most patients with polycythemia vera it is definitely established that leukemia myelofibrosis or some other myelo proliferative disorder develops in an appreciable number perhaps 20 to 30 per cent These complications are more likely to develop in patients with long survival times If the incidence of these disorders is higher in patients treated with P[3] it is possible that the increased incidence is related to the longer survival of the patients so treated as well as to their exposure to some form of irradiation



Figure 37 Extensive subcutaneous hemorrhage follo ing dental extraction in a patient with polycythemia vera

that of 74 per cent found in a much larger series.[38] Myelocytes and metamyelocytes are often present on the blood smear and nucleated red blood cells occur not infrequently. Platelet counts range from 100 000 to 1.5 mil per cu mm.[49] In one series 50 per cent of the platelet counts were above 400 000 per cu mm,[49] and in another 65 per cent were over 300 000 per cu mm.[38]

Study of fixed sections of bone marrow is reported to show a characteristic pattern consisting of clumps of stem cells and early normoblasts in about 85 per cent of patients.[7] Study of smears made from material obtained by aspirations was rarely helpful in our series since about half the marrows appeared to be normal and the remainder showed varying degrees of hyperplasia.[49]

Other laboratory features of the disease are an increased basal metabolic rate, an increased level of uric acid, increased plasma iron turnover,[30] and normal arterial oxygen saturation.[6]

COURSE AND PROGNOSIS

It has been pointed out that the natural history of polycythemia vera is virtually unknown since some form of therapy has always been available.[6] Developments such as thrombosis, hemorrhage and the occurrence of other myeloproliferative syndromes are so frequent that it is difficult to decide whether they should be considered as complications of the disorder or as incidents in its natural history.

A high incidence of vascular thrombosis is to be expected in a disease characterized by an increased blood volume, increased viscosity of the blood, and elevated numbers of platelets when the disease occurs in an age group in which vascular disease is common. The incidence of thrombosis is reported to be 22 per cent,[6] 28 per cent[63] and was found to be as high as 40 per cent in our own series.[49] Thrombosis probably occurs in any of the vessels but appears to be most common in the coronary, cerebral, mesenteric, retinal and peripheral arteries and in the leg, retinal and portal veins. The episodes of thrombosis are seen more often when there is inadequate control of the red cell mass and platelet level by therapy.

Hemorrhage is a frequent occurrence in polycythemia vera. An incidence of 16 per cent,[6] 59 per cent,[63] and 30 per cent[49] has been observed. In our experience the most serious hemorrhages have been intracranial, gastrointestinal and those occurring after various surgical procedures, but extensive ecchymoses, hemarthroses, epistaxis, bleeding after dental extractions and bleeding after minor technical procedures have also proved extremely troublesome.

Peptic ulcer is not uncommon in the disease and the incidence has been found to be from 10 to 14 per cent,[6] 12 per cent[49] and 19 per cent.[38] The incidence of gastrointestinal symptoms is higher, from 17 per cent[49] to 25 per cent,[38] and severe gastrointestinal hemorrhage occurs not infrequently.

The median survival time in polycythemia vera has been reported[38] to be

were once considered to be the treatment of choice such as benzol Fowler's solution phenylhydrazine spray roentgen radiation roentgen irradiation of the spleen and bones phlebotomy and a low iron diet. Some forms of therapy such as splenectomy thyroidectomy and oxygen inhalation never enjoyed much popularity because they were impractical ineffective and even dangerous. Numerous chemotherapeutic agents have been tried in polycythemia and some such as nitrogen mustard, TEM Colcemide pyrimethamine (Daraprim) and 6-mercaptopurine have been found to be inferior to radiophosphorus while at least one Myleran has not been fully evaluated. Bleeding radioactive phosphorus and chemotherapeutic agents are the therapeutic methods that are used most widely at the present time.

Venesection

Venesection is the oldest form of therapy for polycythemia vera. It is a means of reducing the blood volume promptly and often provides prompt symptomatic relief. Because of the development of undesirable symptoms when the blood volume is reduced too rapidly in middle aged and elderly patients it is desirable to remove only 500 ml of blood once or twice a week if the blood volume is to be reduced to normal by this method. Although phlebotomy is an effective means of correcting the blood volume in these patients it is not a satisfactory method of treatment when used alone because platelet levels are not reduced and erythropoiesis is not depressed.

Radioactive Phosphorus

Radioactive phosphorus P^{32} was introduced as a method of treatment for polycythemia vera by Lawrence in 1938[40] and has many advantages for therapy. It can be administered orally or intravenously. It has a half life of 14 3 days and emits only beta rays which travel less than 0 7 cm in tissue.[18] It is concentrated in tissues that have a high rate of phosphorus turnover particularly the bone marrow liver spleen and lymph nodes. In these tissues it produces ionization within the cells and halts cell division. Radioactive phosphorus depresses erythropoiesis myelopoiesis and platelet production which results in a reduction of these elements in the circulating blood. Since radioactive phosphorus does not cause any significant increase in the destruction of mature erythrocytes a significant decrease in the red cell count does not occur for two or three months.

The effectiveness of therapy with radioactive phosphorus is indicated by reports of the reduction in the incidence of thromboses in several large series from 14 8 to 27 4 per cent in patients treated with conventional means exclusive of radiation to 2 7 to 4 2 per cent in patients treated with P^{32} [68] In another series the incidence of thromboses was 50 per cent in 32 patients treated with other forms of therapy and less than 5 per cent in 148 patients treated with P^{32} [9] Comparison of the life expectancy revealed an increase from 7 years in patients treated with drugs phlebotomy and roentgen ray

DIAGNOSIS

In most patients with polycythemia vera the diagnosis is made without difficulty The appearance of the patient the splenomegaly and the increases in the erythrocyte leukocyte, and platelet counts are characteristic Occasionally it is difficult to decide if polycythemia is present in plethoric appearing middle aged hypertensive men with borderline blood counts and it is not easy to determine whether a well established polycythemia is primary or secondary Another problem arises when the leukocyte values are such that the distinction between chronic myelocytic leukemia and a "leukemoid reaction in polycythemia is not easily made

If the hematocrit is 55 per cent and erythrocyte count is elevated to 5 8 to 6 0 mil per cu mm the possibility of an early polycythemia vera cannot be excluded in a middle aged man simply because splenomegaly leukocytosis and thrombocytosis are absent In such circumstances a determination of the blood volume is often helpful

The distinction between primary and secondary polycythemia is usually not a problem because leukocytosis thrombocytosis and splenomegaly do not usually occur in secondary polycythemia In addition the presence of a cardiovascular abnormality or chronic lung disease is usually evident in the secondary type In obscure problems determination of the arterial oxygen saturation is often decisive as the arterial oxygen saturation is almost invariably over 90 per cent in polycythemia vera and is often below this figure when polycythemia is secondary to some condition that produces anoxia

Although the presence of frank myelocytic leukemia either acute or chronic is easily recognized a decision as to when polycythemia vera with a markedly elevated leukocyte count becomes transformed into chronic myelocytic leukemia may be virtually impossible since young cells of the myelocytic series appear in the peripheral blood in both circumstances It has been reported that metabolic differences are demonstrable between the leukocytes in chronic myelocytic leukemia and those seen in polycythemia vera [9] These consist of differences in alkaline phosphatase activity myeloid leukocyte glycogen, and blood histamine These differences suggest that conditions that have been considered to be identical on morphologic grounds may be different metabolically Until more is known about this interesting problem often the best that one can do when faced with this differential diagnosis is to examine the patient periodically before making a decision

TREATMENT

At the present time there is no cure for polycythemia vera Therapy is directed toward reducing the increased blood volume increased viscosity and increased platelet counts Since it has been recognized for many years that these abnormalities are responsible for the clinical manifestations of the disease numerous methods of treatment have been introduced One author lists 24 methods or agents that have been tried [18] Among these are many that

out hospitalization whenever possible Because of the tendency to both hemorrhage and thrombosis that exists in polycythemia vera surgical procedures should be performed only if absolutely necessary Prior treatment of the polycythemia reduces the likelihood of these complications but does not prevent them in all cases

The aim of the treatment of polycythemia vera in our clinic is to keep the hematocrit below 50 per cent and the platelet count below 400 000 per cu mm Initially patients are bled 500 ml once or twice a week until the hematocrit approaches 55 per cent Radioactive phosphorus is considered the treatment of choice and the patient is given a dose of 3 5 to 5 millicuries orally The patient is then observed at monthly intervals If the hematocrit rises appreciably during this interval phlebotomy is performed At the end of four months if the desired results have not been obtained, a second dose of radiophosphorus of 2 to 4 millicuries is administered It is unusual for a patient to need more than two courses of treatment to obtain an initial remission but should it be necessary he receives 2 or 3 millicuries after another four months observation After the initial remission has been obtained patients are followed at regular intervals when possible the frequency depending on the severity and course of the disease

Because of the uncertain danger of an increase in leukemia and other myeloproliferative disorders in patients treated with P[32] and other forms of radiation, control of the disorder by phlebotomy and chemotherapeutic agents such as Myleran is attempted before resorting to radiation in patients less than 40 years of age Should it be established conclusively that radiotherapy does not increase the incidence of leukemia and myelofibrosis or that even in the younger patients the risk of the development of these disorders is small compared to the beneficial effects of this therapy a distinction in the method of therapy based on the age and life expectancy of the patient would not be necessary The same is true if the chemotherapeutic agents whose cellular action is very similar should prove to have late effects similar to those of irradiation Therapy with roentgen rays is rarely used in polycythemia at the present but local therapy over the spleen is a helpful adjunct in patients in the advanced stages who have symptoms due to the marked splenomegaly

SECONDARY POLYCYTHEMIA

A remarkable increase in total red blood cell mass may occur in any situation which results in tissue anoxia Currently it is believed that as a consequence of oxygen deprivation some as yet unidentified oxygen sensitive

to 13.3 years in patients treated with P^{32}.[30] Splenomegaly and hepatomegaly also often respond in a gratifying manner to this form of therapy.

Radioactive phosphorus is administered either intravenously or orally. If given intravenously, about 40 per cent of the dose is lost in the urine in 6 days, and if given orally, about 20 per cent is lost in the feces and an additional 15 per cent is lost in the urine in the same period.[18] It has been found that intravenous or oral doses of 1.5 millicuries do not alter the erythrocyte count in normal individuals.[18] The total dose that has been required to effect a remission has ranged from 2.3 to 21.3 millicuries. The initial dose is usually from 3.0 to 7.0 millicuries and most often from 4.0 to 4.5 millicuries.[9] In about one half the patients a satisfactory remission follows the initial dose, but 40 per cent require a second treatment and 10 per cent require three treatments.[1] In one series the duration of remissions averaged about 2.5 years but sometimes they lasted as long as 10 years.[18] In another report the average interval between courses was about 15 months; however, the greatest number of retreatments occurred in the first 6 to 10 months. After the patient had a satisfactory remission the interval between treatments increased to about 33 months.[38]

It has been strongly suspected that therapy with P^{32} and other forms of irradiation is associated with an increased incidence of leukemia, particularly the acute variety, and other myeloproliferative disorders.[57] Although this important matter is unsettled at the present time,[37] the many advantages of therapy with P^{32} would seem to make it the treatment of choice, certainly in patients above the age of 50 years, since the beneficial decrease in the incidence of thrombosis seems to outweigh by far the danger of the occurrence of leukemia or other allied disorders.

Chemotherapy

Nitrogen mustard (HN_2), triethylenemelamine (TEM), Colcemide and pyrimethamine (Daraprim) have been used in the treatment of polycythemia vera.[17, 18, 3] All have a suppressive effect on the marrow, but the short duration of the remissions that occur and the difficulty in avoiding undesired toxic effects on the marrow have prevented their gaining an important place in therapy. An initial report of the results obtained with Myleran is more promising since this preparation is less toxic than other chemotherapeutic agents and yet produced satisfactory reductions in the erythrocytes, leukocytes and platelets.[64] The danger of these chemotherapeutic drugs as leukemogenic agents has not been determined.

Plan of Treatment

In the treatment of polycythemia vera it is important that the patient be kept ambulatory if possible. If the disease is even moderately severe, bed rest is dangerous for these patients because of the increased likelihood of thrombosis. For this reason patients with the disease should be treated with

If an individual is already compromised by lowered barometric pressure the occurrence of any chronic pulmonary disorder may be catastrophic Marked polycythemia severe cyanosis pulmonary hypertension and right heart strain may result[44] Some degree of improvement usually follows removal to sea level[8 46 8]

Other Causes

In the preceding conditions polycythemia appears to be the result of decreased arterial oxygen saturation, which in some fashion causes the release of a humoral substance capable of stimulating erythropoiesis Polycythemia also occurs in other conditions where the oxygen saturation is normal and the mechanism is unexplained Among these are hypernephroma[21 9] polycystic kidney disease[36] hydronephrosis[11] renal tuberculosis[59] brain tumor[7] hydrocephalus[51] ovarian tumors and uterine myomata[8 6] The suggestion by Jacobson[33] that the kidney may be concerned with the production or metabolism of erythropoietin is interesting in light of the association of varied kidney disorders with polycythemia

THE DI GUGLIELMO SYNDROME

(Erythremic Myelosis Erythroleukemia Erythroblastemia)

Originally described by di Guglielmo in 1923 the characteristic feature of this rare syndrome is marked proliferation of the nucleated red blood cells similar to that of the white cells in leukemia Recent reports have emphasized that not only the erythroid cells but other cell lines as well are involved in the disorder[43 6]

Dameshek[13 14 1] has suggested that the di Guglielmo syndrome be considered together with such diseases as chronic granulocytic leukemia polycythemia vera, and agnogenic myeloid metaplasia as a myeloproliferative disorder and that some undiscovered stimulus may affect all of the cell lines of the marrow to a greater or lesser extent and be responsible for the variable clinical findings characteristic of these disorders In the early phase of the di Guglielmo syndrome red cell proliferation may be the only manifestation of the disorder but if the patient survives myeloblastic proliferation becomes apparent and may predominate

The clinical features of the di Guglielmo syndrome include normocytic normochromic anemia with extraordinary erythroblastic hyperplasia unaccompanied by a commensurate rise in reticulocytes *Atypical forms of nu*

tissue releases the humoral substance erythropoietin[19, 23, 43] Increased erythropoietin is thought to cause an increase in the production of red blood cells by producing an expansion of the marrow volume[29] In secondary polycythemia the increase in the red cell mass is often accompanied by a decrease in plasma volume while in primary polycythemia vera the plasma volume is usually normal In most instances the polycythemia appears to be a physiologic response to decreased arterial oxygen saturation. This response like other homeostatic mechanisms may harm the organism instead of protecting it if sufficiently intense and prolonged Excessive elevations of the red blood cell mass may produce sufficient alterations of the viscosity of the blood to cause circulatory embarrassment and increase the danger of thrombosis[43]

Cardiac and Vascular Malformations

Any cardiac or vascular lesion which results in a high grade veno arterial shunt may produce polycythemia As the result of the mixing of venous and arterial blood inadequately oxygenated arterial blood reaches the peripheral tissues An increase in erythropoietin is thought to occur and stimulate erythropoiesis The subsequent polycythemia increases the oxygen carrying capacity of the blood and affords a measure of relief to the patient

Pulmonary Disorders

Polycythemia occurs in some chronic pulmonary disorders where it appears to be a physiologic response to the decreased arterial oxygen saturation The commonest pulmonary abnormalities that lead to anoxemia are

1 Perfusion of non aerated or poorly aerated alveoli.

2 Altered alveolar membrane characteristics interfering with gaseous exchange

3 Direct pulmonary arteriovenous fistulas

One end result of each of these conditions is decreased arterial oxygen saturation[8] In the first instance the blood flow is through poorly aerated or non aerated segments and there is inadequate exposure of the blood to oxygen In the second instance the pulmonary segments are well aerated but the alveolar membranes have been so altered as to interfere with gaseous exchange between the alveoli and the capillaries Direct pulmonary arteriovenous fistulas cause anoxia because the blood by passes vessels in which gaseous exchange takes place[8]

Low Barometric Pressure

Persons who reside at high altitude with lowered barometric pressure have a decreased alveolar oxygen tension which leads to incomplete arterial oxygen saturation The polycythemia which occurs in response to the anoxia appears to bear a direct relationship to the severity of the oxygen deficit[31]

Four months later the hematocrit had risen to 65 per cent erythrocyte count was 7 3 mil per cu mm and platelets 772 000 per cu mm Five hundred ml of blood was removed by phlebotomy on two occasions to reduce the red cell count and the patient was given an additional 6 0 millicuries of P^{32} by the oral route Two and one half months later the spleen had decreased markedly in size and extended only a single fingerbreadth below the left costal margin.

For the next eighteen months the patient s hematocrit remained between 48 and 50 per cent, platelets less than 500 000 per cu.mm and leukocyte count between 9000 and 13 000 per cu mm At the end of that period however it was found that her hematocrit had risen to 55 per cent, erythrocyte count to 6 6 mil cu mm. and platelet count was 1 200 000 per cu mm The spleen had increased in size and once again extended to the level of the umbilicus The patient was given another 4 millicuries of P^{32} by the oral route and four months later the hematocrit was 51 per cent and platelet count 500 000 per cu mm

The patient then did well from a symptomatic point of view for the next two years despite the fact that the platelet count fluctuated and was sometimes greater than 1 000 000 per cu mm During this time the hematocrit remained less than 48 per cent. Two and one half years after the third dose of P^{32} the hematocrit had risen to 58 erythrocyte count to 7 4 platelets were 1 200 000 per cu mm and the spleen had once again increased in size being 5 or 6 fingerbreadths below the left costal margin At this juncture it was decided to institute therapy with Myleran and observe the effect on the course of the patient s disease Phlebotomy once again reduced her hematocrit and the administration of Myleran 2 mg twice a day was initiated The levels of the cellular elements in her peripheral blood were followed at weekly intervals and were satisfactory After 50 days the Myleran was stopped and three months later the hematocrit was 46 per cent, platelet count 300 000 leukocyte count 9800 per cu mm The spleen was still at the level of the umbilicus The remission has lasted six months

Comments As is true of the majority of patients with polycythemia vera this patient s initial symptoms appeared in middle age The evidence of increased red blood cell mass the presence of a markedly enlarged spleen and generalized marrow hyperplasia suggested the possibility of chronic myelogenous leukemia or polycythemia vera The diagnosis could not be made with certainty on the initial visit The first remission required 10 millicuries of P^{32} and lasted eighteen months Following the administration of an additional 4 millicuries of P^{32} the patient s hematocrit returned to normal levels and she remained free of symptoms for thirty six months longer despite the fact that her platelet count was not adequately controlled When the hematocrit values once again rose to abnormal levels it was decided to use the chemotherapeutic agent Myleran in an attempt to obtain a satisfactory reduction of erythrocytes leukocytes and platelets This was successful and for six months the hematologic values have remained within satisfactory limits

cleated red blood cells may be found in the peripheral blood and bone marrow Some of the nucleated red blood cells appear to be megaloblastic in character but do not respond to the administration of vitamin B_{12} There are a disproportionately large number of immature nucleated red blood cells which increase as the disease progresses [6] The white blood cell count is usually low or normal while the platelets are often diminished It has been reported that careful inspection of blood and bone marrow smears will reveal myeloblastic as well as erythroblastic activity [1]

No therapeutic procedures have proved effective The course is marked by a progressive anemia and leukemia frequently occurs as a terminal event [1]

ILLUSTRATIVE CASES

Case 1

This 53 year old white housewife enjoyed good health until 12 months prior to admission when she had a single profuse nosebleed Six months later she had a fall and examining herself to ascertain its effects noted a non tender mass in the left upper quadrant of her abdomen One month prior to admission she developed dyspnea and ankle edema Examination by her family physician revealed splenomegaly and the patient was referred for investigation

Physical Examination The spleen extended almost to the level of the umbilicus and was moderately firm The liver was palpable 2 fingerbreadths below the right costal margin The remainder of the physical examination was non contributory

Laboratory Studies Erythrocyte count 5 2 mil per cu mm hematocrit 47 per cent leukocyte count 15 000 per cu mm reticulocytes 0 2 per cent Differential count (per cent) myelocytes 1 juvenile 1 bands 10 segmented 79 lymphocytes 4 monocytes 1 eosinophils 3 basophils 1 Platelet count was 426 000 per cu mm Bone marrow examination revealed the marrow to be hypercellular with hyperplasia of all cell lines

It was thought that this patient had either an early polycythemia vera or chronic myelocytic leukemia Treatment was withheld pending further periodic examinations

Treatment and Course Six months later the diagnosis of polycythemia vera was evident She had a very florid complexion the spleen and liver were unchanged The hematocrit was 59 per cent leukocyte count 14 000 per cu mm platelets 580 000 per cu mm The hematocrit was reduced to 55 per cent after three phlebotomies over the next eight days and she was given 4 0 millicuries of P^{32} orally

confusion followed by unconsciousness She remained unconscious and died a few hours after admission Death was attributed to an intracranial hemorrhage

Autopsy showed extensive intracranial hemorrhage There was evidence of old infarction of the myocardium and generalized arteriosclerosis Marked splenomegaly and hepatomegaly were present Microscopic examination of the liver spleen and bone marrow disclosed chronic myelocytic leukemia

Comment The patient died at the age of 78 some ten years after the onset of her symptoms Earlier in her illness she was thought to have myelocytic leukemia rather than polycythemia vera because the hematologic values were obtained after a massive gastrointestinal hemorrhage Later in her course the diagnosis of polycythemia vera appeared to be well established Subsequently the patient had many complications of this disease such as angina pectoris intermittent claudication cerebral thrombosis and postoperative bleeding Her final episode was an intracranial hemorrhage As is often the case it was difficult to determine whether these episodes were due to her blood dyscrasia or to the diabetes mellitus and arteriosclerosis which were present It appeared likely however that the blood dyscrasia was a factor The incidence of complications of this type has been reduced by treatment with radioactive phosphorus and had this agent been used the course of her illness might have been altered Late in her course this patient also developed chronic myelocytic leukemia which was evident at autopsy This development illustrates the unsettled problem of the relationship between polycythemia vera and myelocytic leukemia and the important question as to whether there is any relationship between irradiation therapy of polycythemia and the development of the leukemia

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This patient s course has now extended over seven years during which time she has had no complications and has lived a normal life The difficulty in obtaining a satisfactory reduction in the level of platelets is well illustrated

Case 2

A 69 year old white housewife with known diabetes mellitus of fifteen years duration was admitted with a history of passing tarry stools for four days The most significant abnormalities on physical examination were splenomegaly and hepatomegaly On this admission the hemoglobin was 7 5 gm per 100 ml erythrocyte count was 2 8 mil per cu mm and the leukocyte count was 22 000 per cu mm Differential count (per cent) myelocytes 1 juvenile 1 bands 3 segmented 58 lymphocytes 15 monocytes 21 eosinophils 1 No nucleated red cells were seen Occult blood was present in the stool but barium studies of the entire gastrointestinal tract revealed no intrinsic lesion The sternal marrow was markedly hypercellular and the myeloid erythroid ratio was 3 1

It was thought that the patient probably had chronic myelocytic leukemia with gastrointestinal hemorrhage She was treated with blood transfusions and had a satisfactory response Follow up examinations for several months revealed normal hemoglobin and red count values leukocyte counts of about 17 000 per cu mm and a differential count similar to that noted in the hospital Six months later the patient again had a severe gastrointestinal hemorrhage with melena and hematemesis Following this episode the hemoglobin was 10 5 gm per 100 ml red count 3 3 mil per cu mm leukocyte count 36 000 per cu mm

After recovery from this hemorrhage the red count rose to 6 3 million and hemoglobin to 17 to 18 grams and it was apparent that the diagnosis of polycythemia vera was the correct one Her leukocyte count varied from 12 000 to 36 000 per cu mm Because of the discomfort from the size of the spleen she received several courses of roentgen therapy over the spleen which resulted in a reduction of the splenic size as well as reduction in the leukocytes hematocrit and red cell count

When she was 73 years of age she had angina pectoris and intermittent claudication During the next five years the erythrocyte cell count rose to 7 7 mil per cu mm on several occasions and phlebotomies were performed as indicated She fell and suffered an intertrochanteric fracture of the hip During the operation to pin the fracture there was considerable bleeding two days later a cerebral vascular accident occurred The patient improved slowly and returned home Six months later the patient s hematocrit was 45 per cent erythrocyte count 4 7 mil per cu mm but the leukocyte count had risen to 102 000 per cu mm Differential count (per cent) myelocytes 5 juveniles 8 bands 7 segmented 70 monocytes 8 eosinophils 1 It was the clinical impression that the patient had developed leukemia Three weeks later the patient was admitted to the hospital after an episode of mental

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The defense of hemostasis can then be divided conveniently into a vascular and a biochemical phase. The vascular phase of hemostasis has received little attention when compared to that given the biochemical aspects. The response of the vessel to injury, the role of the vascular endothelium in repair, and the factors responsible for the maintenance of the functional efficiency of the vascular wall are little understood. The importance of the vessel wall in hemostasis is evidenced by the fact that the platelets may be reduced and the blood made biochemically incoagulable without the occurrence of purpura or hemorrhage. This is a promising area for research and deserves more emphasis.[69]

On the contrary, a great deal of information has been gathered concerning the process of blood coagulation. It is with this phase of hemostasis that this section is primarily concerned. While much of our knowledge of blood coagulation is based on empiric laboratory tests, it has nonetheless found extensive clinical application. The theory of blood coagulation to be presented is but an expansion of the classic theory developed at the turn of the century. The various disorders of hemostasis will subsequently be presented within the same framework.

As an aid to the reader, a list of synonyms is given below.[39, 46]

TERMS USED IN TEXT	SYNONYMS
Antihemophilic factor (AHF)	Antihemophilic globulin (AHG) Thromboplastinogen A Factor VIII Platelet co factor I Antihemophilic factor A
Plasma thromboplastin component (PTC)	Christmas factor (CF) Factor IX Autoprothrombin II Antihemophilic factor B Beta thromboplastin
Factor V	Accelerator globulin ACG Pro accelerin Labile factor Co-factor of thromboplastin AC globulin
Factor VII	Serum prothrombin conversion accelerator (SPCA) Pro-convertin Stable factor Co thromboplastin Prothrombin accelerator Autoprothrombin I
Stuart Factor	Stuart Prower Factor Factor X

Blood Coagulation. Theory and Clinical Applications

INTRODUCTION

It is not surprising that the events responsible for hemostasis have been so extensively studied The process by which fluid blood becomes a solid clot has stirred the curiosity of many and the consequences of the failure to maintain hemostasis in a variety of disorders have lent further impetus to the study New techniques for the investigation of these problems have recently become available Application of these techniques has made possible the development of a useful concept of blood coagulation and led to a better understanding of many of the blood dyscrasias which result in abnormal bleeding Advances in therapy have occurred as the result and the clinician is now better able to cope with these distressing disorders

Hemostasis is the end result of a series of related but not necessarily interdependent events Damage to a vessel wall is followed by immediate but temporary reflex vasoconstriction In some manner as yet unknown platelets are attracted to the site of injury and agglutinate During this process of agglutination a vasoconstrictor substance serotonin, is released from the platelets Serotonin affords a more prolonged vasoconstriction of the damaged vessel and causes vasoconstriction of other vessels in the immediate vicinity as well

This initial defense of hemostasis is followed by the formation of a fibrin clot which effectively seals the vessel Later the clot is replaced by connective tissue and undergoes dissolution Repair is then complete

of new factors necessary for the development of normal thromboplastin activity The general design of these investigations is simple An in vitro test system is employed The defect of the plasma serum or platelets of the patient under investigation is identified by successive substitutions of the corresponding fraction of normal blood until the coagulation defect is corrected Thus localized to either the plasma serum or platelets the defect is further identified by comparing the defective fraction with comparable fractions from patients with known defects In this way it can be determined whether the defect under study is the result of the deficiency of a known or previously unknown factor

The result of such studies has been an expansion of the classic theory of coagulation [46]

PRESENT DAY THEORIES OF COAGULATION

For purposes of discussion the coagulation of blood may be considered to occur in three stages During the first stage thromboplastin activity develops as the result both of the coagulation factors in the blood and the admixture of tissue juices and plasma The blood (intrinsic) and tissue (extrinsic) systems responsible for the development of thromboplastin activity may be separated in vitro and shown to be independent and equally potent There seems little doubt that they both function in the physiologic defense of hemostasis The second stage of blood coagulation is the conversion of prothrombin to thrombin The evolution of thrombin from prothrombin will occur in the presence of thromboplastin activity and calcium ions The third stage of blood coagulation is the conversion of fibrinogen to fibrin in the presence of thrombin Each of these stages will be discussed separately

The Development of Thromboplastin Activity

The first stage of blood coagulation may be outlined as follows

Extrinsic (Tissue) System | Intrinsic (Blood) System

Tissue Factors
Factor VII

or

AHF Platelets
PTC PTA
Hageman Factor

\+ +

Factor V
Stuart Factor
Ca^{++}

↓

Thromboplastin Activity

HISTORICAL ASPECTS

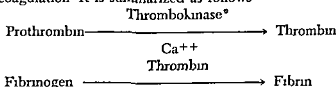
The current concepts of the process of coagulation are better understood when viewed in historical perspective For details of the early history of the study of coagulation the reader is referred to the reviews by Gamgee in 1880[5] and Howell in 1935[34]

In 1845 Buchanan[18] noted that a substance was formed during clotting which when extracted from the clot would cause the coagulation of serous fluids The substance which Buchanan had discovered was thrombin In 1859 Denis [8] demonstrated that a material which was the soluble precursor of the fibrin of the clot was present in the plasma. This protein was named fibrinogen Schmidt,[6] working along similar lines in 1872 concluded that the formation of fibrin was due to the interaction of fibrinogen and serum globulin in the presence of thrombin He noted that the clot promoting substance thrombin could be extracted after a clot was formed but no such substance could be demonstrated in the liquid state From this observation he concluded that thrombin was present in the circulating blood in an inactive or precursor state and the concept of prothrombin was evolved In 1879 Hammarsten [30] using a better method of preparation of fibrinogen demonstrated that it could be converted to fibrin in the presence of thrombin without the participation of serum albumin or globulin

In 1878 the great French hematologist Hayem[3] first demonstrated that the platelets are distinct from leukocytes and suggested that they play a role in coagulation He described the weblike fibrillar structure which occurred during the agglutination of platelets and postulated that this structure reacted with fibrinogen.

In 1890 Arthus and Page proved that calcium was essential for the coagulation of blood It was shown that substances which bind or remove calcium from the blood prevent coagulation

At the turn of the century Morowitz [0] was able to formulate the classic theory of coagulation It is summarized as follows



The classic theory which was simple and agreed with the known facts remained unaltered until recently As additional factors essential to the process of coagulation were discovered expansion of the scheme became necessary but the classic theory remains the basis of most of the modern theories of coagulation

The most important contributions to the theory of coagulation have been concerned with the development of thromboplastin activity The exact nature of the material responsible for the conversion of prothrombin to thrombin is unknown and for purposes of discussion is designated as "thromboplastin activity"

Studies of patients with disorders of hemostasis have led to the discovery

* Thromboplastin activity

of storage at 4 C but may fall off rapidly thereafter [16] Fresh frozen plasma retains its PTC activity for long periods

Plasma thromboplastin antecedent (PTA)[61] was discovered as was PTC during investigation of persons with hemophilia like states It is thought to be a globulin, and migrates with the beta globulins electrophoretically [61a] Since it is not consumed during coagulation it is present in both plasma and serum Unlike PTC it is not adsorbed by inorganic precipitating compounds and is present in $BaSO_4$ adsorbed plasma PTA activity persists in fresh frozen plasma for at least two years and for up to four months in plasma stored at room temperature [9]

The Hageman factor was recognized when a patient was discovered whose blood did not coagulate in a normal period of time on contact with a glass surface The patient had no clinical disorder of coagulation Hageman factor is not adsorbed when plasma is adsorbed with $BaSO_4$ nor is it consumed during coagulation [19 60] It has been suggested recently that the Hageman factor may play a role in the initiation of the clotting process Inhibitors in the blood are believed to keep the factor in an inactive state Exposure to glass activates the Hageman factor which may initiate clotting The active factor is then enzymatically destroyed stopping its action [9]

The platelets contribute to the early phase of coagulation in the intrinsic system [15 31] The disruption of the platelets and subsequent release of their granules seem of great importance The active material in the granules is thought to be ethanolamine phosphatide Platelets also carry serotonin (5 hydroxytryptamine) which is capable of bringing about vasoconstriction The role of this material in hemostasis however is not completely defined.[41 8]

The above named factors are now known to play a role in the early phase of the formation of thromboplastin activity in the intrinsic system New factors will likely continue to be isolated and old factors redefined resulting in a better understanding of coagulation

Additional Factors From this point the extrinsic (tissue) and the intrinsic (blood) systems follow a common pathway resulting in the development of thromboplastin activity Additional factors are necessary for either system to proceed

Factor V is probably formed in the liver but is not vitamin K dependent It is found only in the plasma as it is consumed during the coagulation of blood The time at which it enters the coagulation scheme has been investigated [3] and it seems to play an important part in the later phases of thromboplastin formation Factor V is not adsorbed by inorganic precipitating compounds and remains in $BaSO_4$ adsorbed plasma It migrates between the beta and gamma globulins on filter paper electrophoresis [1]

The Stuart factor[9 13] has but recently had its identity established. It is found in the serum as it is not consumed during coagulation It is adsorbed by inorganic precipitating compounds and is relatively heat stable The Stuart factor has no doubt been confused in the past with Factor VII since the two have many properties in common

The two systems capable of developing thromboplastin activity are initially distinct but utilize a common final pathway Both systems develop equally potent thromboplastin activity though the rate of its development is more *rapid in the extrinsic system* [12]

The Extrinsic System The extrinsic system requires a source of tissue factors *All tissues probably contain the factors essential to the development* of thromboplastin activity and contribute to the physiologic defense of hemostasis Brain or lung tissue is generally used as a tissue source in the performance of coagulation tests For many years it was thought that these tissues contained one active principle but investigation has now revealed that they contain a complex of heat stable and heat labile fractions [46] It is well to recognize that the nature of this complex is not completely understood and that it is in all probability the end result of a series of chemical and enzymatic reactions

Factor VII is one of the plasma factors essential for the development of thromboplastin activity from a tissue source This factor is apparently formed in the liver and like prothrombin is dependent on vitamin K for its continued production Factor VII is not consumed during the coagulation of blood and is found in both plasma and serum It is adsorbed by inorganic precipitating compounds ($BaSO_4$ etc) and is relatively heat stable [5] Factor VII behaves as a beta globulin on electrophoresis It is not considered to play any role in the production of thromboplastin activity by the intrinsic system [2 36 51]

The remaining factors necessary to the development of *thromboplastin* activity by the extrinsic system are essential to the intrinsic system as well *They will be discussed following a description of those factors* unique to the intrinsic system

The Intrinsic System Among the factors essential for the intrinsic system is the antihemophilic factor (AHF) [13 71] so named because a deficiency of this factor is responsible for the bleeding diathesis in classic hemophilia It is a globulin but the site of its production is unknown AHF is relatively short lived in the body with a half life on the order of 8 to 24 hours It is consumed during coagulation and is therefore found only in the plasma [12] AHF is not adsorbed by the inorganic precipitating compounds and is present in $BaSO_4$ adsorbed plasma *The activity of AHF in plasma or whole* blood diminishes rapidly on storage under standard blood bank conditions but will persist when fresh frozen plasma is stored at —30° C [0] It is relatively heat stable AHF has not as yet been obtained in pure form

Plasma thromboplastin component (PTC) was discovered during the investigation of persons with hemophilia like bleeding disorders [3 4 11 13] This factor unlike AHF is not consumed in coagulation and is found both in plasma and serum It is adsorbed by organic precipitating compounds and is not present in $BaSO_4$ adsorbed plasma Though PTC is relatively heat labile it fares somewhat better *on storage at 4° C than does AHF* [3] PTC activity can still be demonstrated in near normal amounts after two weeks

It will be recalled that the conversion of fibrinogen to fibrin in the final phase of coagulation was recognized in 1859 by Denis when he demonstrated the existence of fibrinogen.

Fibrinogen is formed in the liver and is present in the blood in a concentration of 300 mg per 100 ml. Its molecular weight is 450 000 and it is available in a highly purified form.

Fibrin is formed when thrombin is added to fibrinogen.[6, 43, 46, 68] Thrombin has the ability to split arginylglycine bonds of the fibrinogen molecule to form a monomer of fibrin and two peptides A and B.[9, 37] Polymerization of fibrin occurs independently of the presence of thrombin to form a fibrin polymer. Physiologic fibrin is then formed through the action of another plasma substance, at present undefined,[42] in the presence of Ca^{++} ions. The clotting time of fibrinogen is inversely proportional to the concentration of thrombin.

The schematic diagram of the three stages of blood coagulation may now be gathered together and considered as a whole.

Extrinsic (Tissue) System — Tissue Factors, Factor VII

or

Intrinsic (Blood) System — AHF, Platelets, PTC, PTA, Hageman Factor

+

Factor V
Stuart Factor
Ca^{++}

Prothrombin —Thromboplastin Activity / Ca^{++}→ Thrombin

Fibrinogen —Thrombin→ Fibrin Monomer + Peptides A & B

↓

Fibrin Polymer

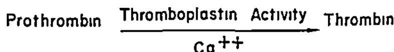
↓ Fibrin Stabilizing Factor, Ca^{++}

Physiological Fibrin

Calcium ions are necessary for the development of thromboplastin activity in concentrations of between 5 and 20 mg per 100 ml Marked prolongation of coagulation time occurs only when the calcium is below 2.5 mg per 100 ml and these levels are not seen clinically The effectiveness of oxalates and citrates as anticoagulants depends on their ability to bind calcium

The Conversion of Prothrombin to Thrombin

The second stage of blood coagulation may be outlined as follows



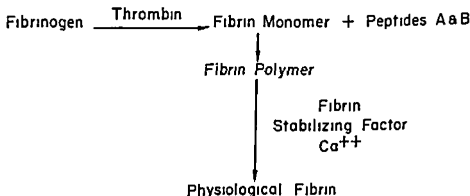
The conversion of prothrombin to thrombin is brought about in the presence of thromboplastin activity and Ca++ ions [6]

The existence of prothrombin was suspected by Schmidt in 1892 and since that time this material has been extensively studied [6] Prothrombin has a molecular weight of 62 700 [38] Eighteen amino acids and hexosamine have been identified in the molecule Glutamic acid aspartic acid and arginine make up approximately 33 per cent of the protein nitrogen On electrophoresis prothrombin behaves as an alpha 1 globulin It is formed in the liver and is dependent on adequate intake of vitamin K for its production

Thrombin is quite similar to prothrombin in its amino acid content and the amount formed in the presence of thromboplastin activity and Ca++ varies directly with the amount of prothrombin available Thrombin is not present in circulating blood It is the material found in shed blood which was shown capable of clotting fluid from serous cavities by Buchanan in 1842 The molecular weight of thrombin is 40 000 [38] A unit of thrombin is defined as that amount of thrombin necessary to clot 1 ml of standard fibrinogen solution in 15 sec at 28 C Thrombin has been highly purified Some of the preparations contain 12 000 units/mg N [66]

The Conversion of Fibrinogen to Fibrin

The final stage of blood coagulation may be considered to occur thus



Recent evidence[10,4,48] indicates that heparin acts in vivo and in vitro by interfering with the development of thromboplastin activity This is suggested by the fact that neither the antihemophilic factor nor Factor V is consumed in its presence There is also direct interference with thromboplastin activity at higher concentrations This antithromboplastin activity may be the main mode of action of heparin though it is known to have an antithrombin effect as well in the presence of albumin [3]

FIBRINOLYSIS

The fibrin clot which is the end product of coagulation of blood must be removed once its function has been served It is thought that the proteolytic enzyme plasmin (fibrinolysin) is responsible for the destruction of the fibrin clot The mechanism by which the inert form of plasmin (plasminogen) is converted to the active enzyme probably entails a number of steps and is not completely understood [6] It is known to occur spontaneously and in response to fear exercise and trauma [4] It has been suggested that certain types of lipids present in the plasma may affect the mechanism [63]

EXAMINATION OF THE PATIENT WITH HEMORRHAGIC DIATHESIS

The most accurate clinical diagnosis in the case of hemorrhagic diathesis is reached when a specific defect is demonstrated and its cause determined To attain this goal special laboratory techniques are often required in the course of the diagnostic examination which begins when the patient consults his physician because of abnormal bleeding As with any diagnostic problem however the physician notes the character of the symptoms and signs then proceeds in an orderly manner to a consideration of the various disorders known to produce such complaints With constant review of the evidence collected during the course of his examination the physician is able to exclude certain disorders while pursuing others with increased interest Final identification of the cause of the complaint allows institution of rational therapy

History

It is sometimes difficult to establish whether a particular patient has a hemorrhagic diathesis or not The answer may be obvious if the patient presents with purpura or hemarthroses but may be elusive when a patient with a history of "easy bruising is to be evaluated prior to elective surgery The presence of a hemorrhagic diathesis is best demonstrated by the history as results of laboratory tests are often variable during the course of these disorders A history of abnormal bleeding is far more significant than a battery of negative laboratory tests

No theory of blood coagulation may be considered fact until the chemical reactions responsible for the coagulation of blood are elucidated. The one presented is but an expansion of the classic theory and remains empiric. Deletions or additions must be made as old factors are reconsidered and new factors make their appearance. Despite its defects this theory serves as a useful clinical and laboratory guide.

PHYSIOLOGIC ANTICOAGULANTS AND FIBRINOLYSIS

The mechanisms responsible for keeping the blood fluid within the vascular system have not been clearly defined. It seems reasonable that there must be a delicate balance between those forces tending to produce coagulation and those tending to inhibit it. There must also be measures by which the body can dispose of a clot once repair of a vessel is complete. Failure of the blood to remain fluid within the vascular system results in a local thrombosis which may then be the source of emboli to other parts of the vascular system. A thrombosis may bring sudden death to any patient forced to inactivity by disease, surgery, or childbirth. Studies of this aspect of coagulation are of considerable importance in all fields of medicine and there are few fields in which new methods of approach are more urgently needed.

Antithrombin

Thrombin has been noted to be an extremely potent proteolytic enzyme and must be removed or in some manner neutralized following physiologic coagulation or all of the blood in the body would soon be clotted. There are one or more substances which may bring about progressive inactivation of thrombin. Partial purification of one such substance[44] has revealed it to be a globulin. This antithrombin forms a stable compound with thrombin which has been termed metathrombin. Another antithrombin is thought[3] to be present in the albumin fraction of the serum. The action of this latter substance is enhanced by heparin.

Thrombin is also adsorbed by fibrin and thus inactivated. Still other antithrombin activities have been postulated.[64]

Heparin is a mucopolysaccharide which is similar to the chondroitin sulfuric acid of cartilage. It was isolated from the liver of dogs and contains large quantities of glucuronic acid (26 per cent) and glucosamine (23 per cent).[3] Heparin has not been isolated from tissues of the human body and its presence in the circulating blood of man is disputed.

Heparin is used clinically as an anticoagulant and its action may be quantitatively neutralized by protamine.[19] It is interesting that this reaction with protamine was discovered by investigators searching for a material to prolong the action of heparin. They chose protamine because of its action in slowing the release of insulin from subcutaneous depots.

Recent evidence[10, 4, 48] indicates that heparin acts in vivo and in vitro by interfering with the development of thromboplastin activity. This is suggested by the fact that neither the antihemophilic factor nor Factor V is consumed in its presence. There is also direct interference with thromboplastin activity at higher concentrations. This antithromboplastin activity may be the main mode of action of heparin, though it is known to have an antithrombin effect as well in the presence of albumin.[53]

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The history obtained from a patient suspected of having a blee diathesis should begin with a detailed review of the events of his birth early childhood Many disorders of hemostasis are familial and manife soon after delivery Specific inquiry should be made as to the occurr of excessive bleeding from the umbilical cord or following circumcis Prolonged bleeding or excessive bruising in the wake of minor trauma sho be regarded with suspicion Tonsillectomy and adenoidectomy are c lenges to which many children are subjected and to which a child wi *hemorrhagic diathesis may respond abnormally Occasional epistaxis is* unusual in childhood but repeated or severe epistaxis is cause for conc Loss of the first teeth during childhood is often accompanied by slight ble ing but persistent bleeding is evidence in favor of a hemorrhagic diath

On reaching adult life the hemostatic defenses of the patient conti to be challenged The female patient may note excessive menstrual bleed Pregnancy and subsequent delivery further test hemostasis In the ev that excessive bleeding occurs with delivery or menstruation eff should be made to determine whether some obstetric or endocrine c dition rather than a defect in hemostasis could be responsible for bleeding Minor cuts and scratches sustained in the kitchen or gard constantly test the efficiency of hemostasis Operative procedures constit important diagnostic trials of hemostasis and the result of each should recorded The oozing of blood from the gums after brushing the teeth occ occasionally in normal persons but is of significance if severe or persiste Men are daily exposed to minor razor nicks and will remember excess bleeding because of the inconvenience it causes It is clear that the m important tests of hemostasis are those afforded by the stress of day to d life about the home or while at work The results of these tests are availal to the *physician for the asking*

The family history is of paramount importance in determining if t defect is congenital or acquired It is essential to discover whether a members of the patient s family are known to be "bleeders" If it appe *likely that others do have a hemorrhagic diathesis the physician shou* then examine as many members of the family as are available This is impo tant not only to the patient at hand but for purposes of counseling membe of the family group Such studies will allow the physician the opportuni of contributing to the general understanding of the genetic *mechanism* involved

The onset of abnormal bleeding at birth or during infancy is stror evidence that the disorder was genetically transmitted and a negative fami history does not rule out the possibility Some disorders of coagulation a transmitted by genes that are autosomal recessives and the patient must b homozygous for the abnormal gene in order that its presence be clinicall apparent The child in question may be the first member of the famil homozygous for the abnormal gene or may represent a mutation The patien

may be assumed to have an acquired hemorrhagic diathesis if he has reached adult life before the onset of abnormal bleeding and has a negative family history

Physical Examination

Once it is established that the patient has a defect in hemostasis it is important to determine what phase of hemostasis is defective and if the hemorrhagic diathesis is of the acquired type the nature of the underlying disease The physical examination with particular reference to the characteristics of the abnormal bleeding will usually provide helpful information

A disorder of the vascular phase of hemostasis is suggested by the finding of petechiae ecchymoses and superficial bruising Characteristically these lesions are found in the skin of the extremities and over pressure points The most common sites are the lower legs and ankles but the forearms wrists and buttocks are frequently involved Petechiae may also be seen in the mucous membranes of the mouth and on ophthalmoscopic examination of the retina. The ecchymotic lesions of senile purpura are located over the dorsum of the hands and feet where there is but scant subcutaneous tissue to provide support for the vessels The occurrence of purpuric lesions only in the skin of the hands feet and ears suggests the presence of a cold precipitable protein or cold agglutinin as these areas are most subject to exposure to the elements Petechial hemorrhages widely scattered over the trunk as well as the extremities may be the result of a rickettsial bacterial or viral infection A clue as to the etiology of the purpura may be found in the associated dermatologic lesions The Schoenlein Henoch syndrome is characteristically associated with a variety of skin lesions and erythema edema and necrosis of the skin have all been noted The presence of eczema hives or wheals may indicate that an allergy is the inciting cause of the purpura while the finding of purplish red infiltrations of the skin associated with petechiae and ecchymoses might point to a leukemic process

A disorder of blood coagulation is suspected in the presence of joint hemorrhage large hematomas and post traumatic hemorrhage The joint into which bleeding has occurred is painful and swollen and the presence of fluid is usually demonstrable Later there may be ankylosis and residual bone destruction All joints should be carefully examined for evidence of prior hemorrhage Hematomas may develop at any site but occur most frequently in those parts of the body exposed to trauma Truly spontaneous hemorrhage is very rare but the amount of trauma necessary to produce hemorrhage may be slight indeed It has been reported, for example that hemorrhage into a vocal cord may occur while a patient is singing Common locations of deep hematomas are the tongue neck, buttocks and extremities The persistence of bleeding following operative or accidental trauma or its recurrence should alert the physician to the possibility of a bleeding dyscrasia

Other details of the physical examination are also important Ophthalmoscopic examination of the retina may reveal retinopathy compatible with the presence of leukemia thrombocytopenia anemia or polycythemia Of these only the lesions of leukemia are specific The perivascular sheathing of the retinal veins seen in this disorder is thought to represent collections of leukocytes Subhyaloid flame shaped or punctate hemorrhages may be seen in patients with severe anemia or thrombocytopenia Distention of the veins and a diffuse plethora of the retina are often present in polycythemia The presence of hemorrhagic lesions with pale centers is suggestive of endocarditis though similar lesions may occur in anemia and in tuberculosis

The gums of patients with leukemia may be swollen and oozing blood Ulcerations of the buccal mucosa and posterior pharynx are also commonly associated with this disease The mouth is also a frequent site of the lesions of hereditary hemorrhagic telangiectasia

Laboratory Examination

Following completion of the clinical examination of the patient the physician must turn to the laboratory for further information as to the nature of any defect in hemostasis which he has uncovered It is important that he understand just what information laboratory tests do and do not afford The tests which are used to identify defects in hemostasis are empiric The reagents used in the performance of the tests are not pure and the reactions involved are largely unknown Their usefulness is directly related to the experience of the technical staff who must perform the tests and the ability of the physician to interpret them

The technical staff must be familiar with the range of normal results in their own laboratory Results considered to be within the normal range in one laboratory may not be so considered in another The constant use of normal controls is essential if meaningful results are to be obtained The physician should become thoroughly familiar with the laboratory tests available to him He should know something of the nature and purity of the reagents used the technique of performing the tests and the calculations used to derive the results recorded in the chart He will then be able to interpret correctly the results of these empiric procedures and so obtain much useful information

The platelet count bleeding time tourniquet test and estimation of retraction all measure the adequacy of the vascular phase of hemostasis The *platelet count* is quantitative and affords information as to the number of circulating platelets [1] The factor used in calculating the result of the indirect count is from 2500 to 5000 and that used in the direct count from 1000 to 1500 A variation of 10 platelets counted by either method may result in a difference of 10 000 to 50 000 platelets per cubic millimeter being recorded in the chart A careful examination of the peripheral blood smear

often affords as much information as the quantitative enumeration of the platelets

The *bleeding time* is designed to test the response of small vessels to injury[29] It is immediately apparent that variations in the thickness of the skin and the distribution of the vessels combine to prevent uniformity of the test from patient to patient or indeed in the same patient from day to day It is likely that the Ivy method of obtaining the average bleeding time of three uniform punctures of the skin of the inner surface of the forearm has some advantage over that obtained from the more commonly used single ear lobe puncture Again it should be pointed out that the reliability of this test is directly related to the experience of the person doing it False positive as well as false negative results may be obtained

The *tourniquet test* is perhaps less reliable than either the platelet count or the bleeding time Designed to test the response of the small vessels to stress its reproducibility is depressingly rare Minor variations from normal are unreliable and major variations can in many instances be predicted in advance When it seems obvious that the tourniquet test will be positive the performance of the test with the attendant discomfort to the patient is perhaps unwise

Both the speed and completeness of *clot retraction* have been correlated with the quantity and quality of the platelets in the blood In addition to reflecting alterations in platelet number and function clot retraction is affected by the red blood cell volume Clot retraction is also influenced by certain disease states among which are multiple myeloma pneumonia and jaundice[1 47]

The one stage *prothrombin time* is used to reveal deficiencies of those factors essential to the conversion of prothrombin to thrombin in the presence of *tissue* thromboplastin activity The factors which influence the results of the test are Factor V Stuart factor Factor VII and prothrombin Since the end point of the test is the formation of a fibrin clot a deficiency of fibrinogen will also be revealed This test despite its lack of specificity still is of great clinical importance [5]

The *coagulation time* is another non specific test which measures the over all effectiveness of blood coagulation[40] It is a rather insensitive test and moderate abnormalities of the first stage of blood coagulation may go undetected It is most accurately performed by the Lee and White four tube method Care should be taken that an admixture of tissue juices with the blood does not occur during venipuncture

The Thromboplastin Generation Test The thromboplastin generation test has proved to be one of the most important tests of blood coagulation[9] It will reveal any deficiency of the coagulation factors essential to the development of thromboplastin activity in the intrinsic (blood) system The test is based on the fact that potent thromboplastin activity develops during the incubation at 37 C of normal $Al(OH)_3$ adsorbed plasma (containing

AHF PTA Hageman and Factor V) normal serum (containing Stuart PTA Hageman and PTC) platelets or a cephalin substitute together in the presence of calcium ions The rate of development of the thromboplastin activity is measured by removing a sample of the incubation mixture at one minute intervals and measuring the time required for it to produce coagulation of normal platelet free citrated plasma The rate of development of thromboplastin activity in incubation mixtures using normal components is then compared to the rates obtained when the patient s plasma serum or platelets are substituted serially for their normal counterparts in three additional incubation mixtures Each of the three mixtures contains two normal components and one from the patient Thus the defect may be localized in the serum adsorbed plasma or platelets

If the development of thromboplastin activity is abnormal when the patient s serum is substituted for the normal serum in the incubation mixture the defect could be considered to be any one of the factors found in normal serum (Stuart factor PTA Hageman or PTC) Both PTA and Hageman factor are also present in $Al(OH)_3$ adsorbed plasma and as they would be contributed by the normal adsorbed plasma in the mixture their absence in the patient s serum would not be revealed Thus the defect must be considered to be either of PTC or Stuart factor As Stuart factor is essential to the one stage prothrombin time test and PTC is not they can be separated by this test

Should the development of thromboplastin activity be decreased when the patient s $Al(OH)_3$ adsorbed plasma is substituted for normal adsorbed plasma in the incubation mixture the defect would be considered due to a deficiency of one of the factors contained in normal adsorbed plasma (AHF Factor V PTA or Hageman) A deficiency of PTA or Hageman is ruled out as they would be contributed by the normal serum in the incubation mixture The defect must then be one of AHF or Factor V Once again the one stage prothrombin time test serves to differentiate the two as Factor V is essential for the normal evaluation of thrombin in the test and AHF is not

PTA and Hageman factor are present both in normal serum and in $Al(OH)_3$ adsorbed plasma Should the patient have a deficiency of either factor it will not be revealed if normal adsorbed plasma or normal serum is in the incubation mixture However when the patient s serum and $Al(OH)_3$ adsorbed plasma are used together in the incubation mixture in place of their normal counterparts the thromboplastin generation will be abnormal [7, 8] There remains the task of separating Hageman factor deficiency from that of PTA This can usually be done on clinical grounds as patients with Hageman factor deficiency have no hemorrhagic diathesis Furthermore the addition of PTA deficient plasma or serum will correct the clotting defect of Hageman factor deficiency and vice versa This however requires the availability of abnormal serum or plasma

Circulating anticoagulants may produce abnormal results in the thromboplastin generation test when either the patient's serum or adsorbed plasma is substituted in the incubation mixture. If the titer of circulating anticoagulant is sufficiently high, both the patient's serum and adsorbed plasma will produce abnormal results. As circulating anticoagulants are not adsorbed by inorganic precipitating agents the use of 50 per cent normal and 50 per cent patient's adsorbed plasma and normal serum in the incubation mixture will also produce abnormal results. As the results of the thromboplastin generation test in the case of Factor V or AHF deficiency would be corrected by such an addition of normal adsorbed plasma this maneuver may be used to identify the presence of circulating anticoagulants.

Should the platelets of the patient be deficient in factors necessary for the development of thromboplastin activity it will be apparent when an equal number of platelets from the patient and from a normal donor are compared under conditions of the test.

Prothrombin, Factor VII, fibrinogen, Factor V, and Stuart factor are all essential for the normal evolution of thrombin from prothrombin in the presence of *tissue* thromboplastin activity. A deficiency of any of these factors results in a prolonged one stage prothrombin time. If a patient has a prolonged one stage prothrombin time and the results of the thromboplastin generation test are normal, Factor V deficiency and Stuart factor deficiency are removed from consideration. A thrombin titer will determine whether a fibrinogen deficiency is responsible for the abnormal results of the test. To separate Factor VII deficiency from prothrombin deficiency a modification of the thromboplastin generation test may be employed. The patient's plasma is used as a substrate to test the thromboplastin activity of a normal incubation mixture. It has been shown that the substrate clotting time in the presence of normal intrinsic thromboplastin activity depends on the concentration in the substrate of prothrombin and fibrinogen. A normal clotting time precludes a prothrombin deficiency.[33, 6]

Fibrinogen Concentration. The thrombin time and titer are used to estimate the concentration of fibrinogen in a patient's plasma. The thrombin used is that which is commercially available. It is made up according to the manufacturer's instructions and should be adjusted so that 0.1 ml of thrombin will give a clotting time of 10 to 12 seconds when added to 0.25 ml normal plasma. Platelet free citrated plasma from the patient and a normal control are prepared by centrifuging citrated whole blood at 2000 rpm for 15 minutes. Serial dilutions of both normal and patient's plasma are made and the highest dilution which contains a visible clot after addition of 0.1 ml of thrombin is determined. Normal plasma should still contain a clot when diluted 1/64 or 1/128. The thrombin time is the thrombin clotting time of the 1/2 dilution of patient's plasma compared to that of a normal. The objection to using whole blood for these determinations is that it is difficult to see the clot. This test also has the advantage over those which estimate

the quantity of fibrinogen biochemically in that what is tested is reactive fibrinogen while the biochemical assay may include inert fibrinogen or other proteins[67]

Acquired hypofibrinogenemia often presents as an obstetrical or surgical emergency and the equipment necessary to perform the simple tests required for the diagnosis should be convenient to the operating theater and the delivery rooms Failure of shed blood to form a firm clot should immediately alert the attending physician to this emergency As acquired hypofibrinogenemia may be associated with Factor V and AHF deficits as well treatment must remedy these defects as well as replace the fibrinogen

Choice of Laboratory Tests These laboratory tests are sufficient to obtain the information needed to identify the particular defect responsible for disordered hemostasis in the vast majority of patients There are many excellent laboratory procedures available which might serve to augment or extend the results obtained by those presented but space does not permit their inclusion For details of other methods the reader is referred to texts devoted entirely to the subject of blood coagulation[14 54 0]

The physician must determine which of these tests is necessary on the basis of his clinical findings If the clinical examination gives no clue as to which phase of hemostasis is at fault the platelet count bleeding time tourniquet test coagulation time and one stage prothrombin time should be obtained This group of tests affords information concerning both phases of hemostasis and if the results of each are normal it is unlikely that the patient has a disorder of hemostasis The coagulation time however is an insensitive test and the thromboplastin generation test will be needed to detect a mild abnormality of the first stage of coagulation

Should the results of the clinical examination suggest a disorder of the vascular phase of hemostasis the platelet count tourniquet test bleeding time measurement of clot retraction coagulation time and one stage prothrombin time should be obtained The coagulation time and one stage prothrombin time should be within normal limits if only the vascular phase of coagulation is abnormal The tourniquet test should be positive and the bleeding time prolonged. The platelet count may be depressed and clot retraction may be poor or not occur at all If the bleeding time alone is abnormal a thromboplastin generation test should be obtained to be sure that no deficiency exists in the first stage of coagulation

If the physician feels that the biochemical phase of hemostasis is at fault the platelet count bleeding time one stage prothrombin time and thromboplastin generation test should be obtained The platelet count and bleeding time should be normal If the one stage prothrombin time is prolonged and the thromboplastin generation test reveals no deficiency of Factor V or Stuart factor it must be determined if diminished prothrombin fibrinogen or Factor VII is responsible A thrombin time will establish the adequacy of fibrinogen concentration and a modification of the thromboplastin generation test will separate Factor VII and prothrombin

Table 8 notes the results of various laboratory tests in disorders of hemostasis

Table 8 Tests of Hemostasis

Phase	Stage	[illegible]	Platelet [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Thromboplastin [illegible] Serum	Plasma	Platelets	Serum and Plasma	[illegible]	[illegible]	[illegible]
...scular Phase of ...ostasis		Thrombocytopenic Purpura	A	A	A	A	N	N	N	N	N	N	N	N
		Non-thrombocytopenic Purpura	N	A	N	A	N	N	N	N	N	N	N	N
		Hereditary Hemorrhagic Telangiectasia	N	N	N	N	N	N	N	N	N	N	N	N
...chemical Phase of ...mostasis	First Stage of Clotting	AHF Deficiency	N	N	N	N	/	N	A	N	A	N	N	N
		PTC Deficiency	N	N	N	N	/	A	N	N	A	N	N	N
		PTA Deficiency	N	N	N	N	/	N	N	N	A	N	N	N
		Circulating Anticoagulants	N	N	N	N	/	/	/	N	/	/	/	N
	First and Second Stage of Clotting	Stuart Factor Deficiency	N	N	N	N	/	A	N	N	A	A	N	N
		Factor V Deficiency	N	N	N	N	/	N	A	N	A		N	N
	Second Stage of Clotting	Prothrombin Deficiency	N	N	N	N	/	N	N	N	N			N
		Factor VII Deficiency	N	N	N	/	N	N	N	N	N	A	N	N
	Third Stage of Clotting	Congenital Afibrinogenemia	N	N	A	N	A	N	N	N	N	A	A	A
		Acquired Hypofibrinogenemia	/	N	A	N	A	/	N	N	/	A	A	A
...scular and ...chemical Phase of ...mostasis		von Willebrand's Disease	N	N	N	A	N		A	N	A	N	N	N

N — Normal A — Abnormal A — Inconstant

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CHAPTER VII

Disorders of Hemostasis

THE VARIOUS clinical syndromes which occur as the result of disordered hemostasis have been defined largely by application of a series of empiric laboratory tests

Incomplete understanding of the biochemical reactions involved in these tests makes impossible a definitive classification of the disorders of hemostasis at this time For purposes of discussion they will be presented within the framework of the theory presented in Chapter VI and grouped according to the results of the major laboratory tests in common use

Classification

I Vessel and or Platelet Abnormalities
  A Purpura
    1 Thrombocytopenic purpura
      a Secondary
      b Idiopathic
      c Neonatal
      d Thrombotic
    2 Non thrombocytopenic purpura
      a. Allergic

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The level below which the platelets must fall before bleeding occurs varies from person to person. Bleeding is rare when the platelet count is above 50,000 per cu mm but it may not occur even when the platelets fall below this level. The life span of the platelet has been variously estimated at from 3 to 10 days. If a strict balance between production and destruction is to be maintained, 10 to 30 per cent of the circulating platelets must be replaced daily.

Thrombocytopenia, like anemia or fever, should be considered as a symptom of disease, not as a disease in itself. Every effort must be made to determine the cause.

Pathologic Physiology of Thrombocytopenia

The physiologic function of the platelets has been extensively investigated[109, 1, 12, 138, 14, 14] but as yet there is only incomplete understanding of their role in hemostasis. They are known to participate in both the vascular and biochemical defense of hemostasis. They mechanically plug rents in the wall of a vessel by their agglutination and thrombus formation. At the same time they release serotonin, a powerful vasoconstrictor substance.

Platelets are essential to the normal evolution of thromboplastin activity in the intrinsic or blood system and in the final phase of coagulation they influence clot retraction by their interaction with fibrin. They have also been demonstrated to contain a heparin neutralizing factor.

It is apparent that a quantitative decrease in cells which play an important mechanical role in hemostasis and participate in at least two of the three major phases of blood coagulation would represent a serious hazard to the organism. It is not readily apparent, however, why relatively comparable degrees of thrombocytopenia are associated with such variability in clinical findings. It has been speculated that the plasma may contain a factor that is essential to the maintenance of vascular integrity and is complementary to the mechanical role of the platelet. The importance of such a factor in hemostasis would be enhanced if the platelets were diminished.[5]

The disturbed laboratory tests and clinical findings presented by patients with thrombocytopenic purpura may be ascribed to a defect in hemostasis resulting from a quantitative decrease in platelets in association with some as yet undefined change in the vascular endothelium.

Clinical Examination

Characteristically, the clinical manifestations of thrombocytopenic purpura from any cause develop over a period of several days or weeks, though the onset may on occasion be sudden. Bruising or prolonged bleeding from minor trauma, menorrhagia, or epistaxis may be noted before the appearance of petechiae which are the hallmark of the thrombocytopenic state. Petechiae vary in size from those too small to see with the unaided eye to approximately 1/16 inch in diameter. They are the result of tiny extravasations of

b Infectious
c Other
B Hereditary Telangiectasia
II Abnormalities of Blood Coagulation
A Disorders affecting predominantly the first stage of blood coagulation and resulting in an abnormal thromboplastin generation test
1 Antihemophilic factor deficiency
2 Plasma thromboplastin component deficiency
3 Plasma thromboplastin antecedent deficiency
4 Circulating anticoagulants
B Disorders affecting both the first and second stage of blood coagulation and resulting in an abnormality of both the thromboplastin generation test and the one stage prothrombin time
1 Factor V deficiency
2 Stuart factor deficiency
C Disorders affecting predominantly the second stage of blood coagulation and resulting in an abnormal one stage prothrombin time with a normal thromboplastin generation test
1 Factor VII deficiency
2 *Prothrombin deficiency*
D Disorders affecting the third stage of blood coagulation resulting in an abnormal one stage prothrombin time and qualitative or quantitative changes in fibrinogen
1 Congenital afibrinogenemia
2 Acquired hypofibrinogenemia
III Disorders of both vessel function and blood coagulation
A Von Willebrand s disease
B Thrombopathia

I VESSEL AND/OR PLATELET ABNORMALITIES

THE PURPURAS

The term purpura originated from the Greek word *porphyra* which was the name of the mollusk from which a purple dye was obtained [67] Prior to the sixteenth century purpuric lesions were thought to occur only during the course of fevers" such as plague or typhus From that time however it has been recognized that purpura may occur in the absence of a febrile illness

The conventional classification of the purpuras into those associated with thrombocytopenia and those with quantitatively normal platelets seems justified The difference is striking The significance of this difference is not at all clear as will become apparent from the discussion

THROMBOCYTOPENIC PURPURA

The relationship between a decrease in the number of platelets in the peripheral blood and the occurrence of spontaneous bleeding is well known

Table 9 Causes of Secondary Thrombocytopenic Purpura

1 *Invasion of the marrow cavity with subsequent suppression or destruction of the normal marrow elements by*
  a. Malignant cells Carcinoma, sarcoma, leukemia, lymphoma
  b Granuloma Sarcoid, tuberculosis
  c Lipoidosis Gaucher's disease, reticuloendotheliosis
  d. Microorganisms Bacteremia, viremia
2. *Direct suppression of marrow elements by*
  a Physical agents roentgen rays radioactive isotopes
  b Chemical agents
    (1) Universal susceptibility Urethane alkylating agents (nitrogen mustard Myleran, chlorambucil, TEM) benzol antimetabolites (a methopterin aminopterin 6-MP)
    (2) Individual susceptibility
      (a) Drugs Any from the group of analgesics antipyretics antibiotics, chemotherapy antihistamines hormones
      (b) Food
      (c) Insecticides DDT
      (d) Dyes Organic
      (e) Other Any new agents to which the patient has been exposed must be suspected
3 *Peripheral destruction of platelets by*
  a Excessive demands from abnormal coagulation
    (1) Widespread intravascular clotting
    (2) Burns
  b Hypersplenism enlarged spleen from any cause
  c Hypothermia and heat stroke
  d Hemangioendothelioma
  e Blood transfusions
    (1) Incompatible immunologic incompatibility of platelets as well as red cells trapping and stasis intravascular coagulation
    (2) Compatible after large numbers of transfusions Mechanisms unknown
  f Viremia and bacteremia
  g Collagen disorders

While thrombocytopenic purpura per se is a serious condition the ultimate prognosis for any given patient depends largely on the underlying cause Thrombocytopenic purpura following measles threatening as it may be does not carry so grave a prognosis as that associated with a malignancy

MECHANISMS Thrombocytopenia is the end result of increased destruction of platelets decreased production of platelets or a combination of the two The means through which this result is reached varies with the inciting cause The decrease in platelets which occurs as the result of the invasion of the bone marrow by tumor cells granuloma, the reticuloendothelioses lipidoses and microorganisms is thought to be the result of (a) physical crowding of normal megakaryocytes (b) competitive utilization of nutrient substrate by the invading cells or (c) production of metabolic end products by the invading cells which are toxic to the normal marrow elements Thrombocytopenia that follows exposure to ionizing radiation and those chemicals which universally depress bone marrow function is believed due to the interference of those agents with cellular division perhaps through inhibition of deoxyribonucleic acid synthesis [30, 89] Vitamin B_1 folic acid and ascorbic

blood from the arteriolar end of the capillary loop in the skin or mucous membranes[63] A coalescence of petechiae results in larger lesions of varied size which are referred to as ecchymoses

Purpuric lesions may occur anywhere in the body Those located in the mucous membranes are not as well supported as those in the skin and free bleeding into the genitourinary tract or the gastrointestinal tract is common The total amount of blood found in the urine or feces is more likely the result of multiple tiny hemorrhages than of a large hemorrhage from a single petechia The occurrence of bleeding within the central nervous system presents the most serious threat Once again it appears that the failure of the supporting tissues to contain the petechial hemorrhage allows its progression to serious proportions The occurrence of bleeding in the optic fundus is not infrequent and may result in permanent impairment of vision

The physical findings will vary depending on the organ system involved and the magnitude of the blood loss

Laboratory Examination

Thrombocytopenia is the *sine qua non* of this condition The degree of depression of the platelet count varies and is not always related to the severity of the clinical manifestations If symptoms are present however the platelet count is usually below 40 000 to 50 000 per cu mm Anemia is not commonly noted in these patients If present it is normochromic and normocytic in type and the degree of the anemia usually reflects the magnitude of the bleeding It is unusual for the platelets to be sufficiently depressed to produce an alteration in the clotting time The bleeding time is prolonged but may vary from day to day The results of the test may return to normal during the course of the disease though the platelet level remains depressed Clot retraction is poor and indeed there may be no retraction of the clot after 24 hours The degree of clot retraction is usually directly proportional to the number of platelets present The tourniquet test is positive but the performance of the test is usually unnecessary and unwarranted in the presence of thrombocytopenic purpura

Clinical Syndromes

Four forms of thrombocytopenic purpura have been described (a) Secondary thrombocytopenic purpura (b) idiopathic thrombocytopenic purpura (c) neonatal thrombocytopenic purpura (d) thrombotic thrombocytopenic purpura

As there is considerable variation in the etiology course prognosis and treatment of these disorders they will be discussed separately

(a) Secondary Thrombocytopenic Purpura Secondary thrombocytopenic purpura bears a clear and identifiable relationship to some primary agent or disease Some of the diseases and chemical and physical agents that may result in secondary thrombocytopenic purpura are listed in Table 9

penia Precipitating causes are legion Exposure to any new drug chemical or foodstuff must be suspect The possible association with recent infection should be kept in mind Accidental as well as therapeutic exposure to ionizing radiation must be looked for in view of the widespread use of nuclear fission products in industry and medicine A careful history and physical examination will often reveal the underlying disease

Treatment of secondary thrombocytopenic purpura will depend in large measure on the nature of the underlying disorder In addition to therapy directed at the precipitating cause therapy to relieve the bleeding diathesis may be indicated and will be discussed under idiopathic thrombocytopenic purpura

(b) Idiopathic Thrombocytopenic Purpura The separation by Werlhof of the morbus maculosus hemorrhagicus" from the infectious diseases in 1735 was the first recognition of this disorder as a distinct entity It was not until 1883 that Krauss recognized that the platelets of these patients were deficient in number[6] Kaznelson suggested the use of splenectomy in the treatment of these patients in 1916

As the term "idiopathic indicates knowledge of the etiology of this disorder is still incomplete and the diagnosis is made only after exclusion of the diagnosis of secondary thrombocytopenic purpura

INCIDENCE In a group of 50 patients with idiopathic thrombocytopenic purpura seen at the University of Virginia Hospital in the past ten years one half were under 20 years of age Of the remaining 25 patients 9 were past 50 and 3 were past 60 at the onset of their illness While other series[5 6] indicate a similar preponderance in youth the occurrence of the disease in so many patients beyond 50 years of age is unusual There was no significant sex difference in our series though idiopathic thrombocytopenic purpura has often been reported to be more common in females than in males[5 56]

MECHANISM A great deal of effort has been made to elucidate the factors responsible for the development of idiopathic thrombocytopenic purpura The finding that an altered immunologic response was the basis of some of the drug induced secondary thrombocytopenic purpuras spurred efforts to demonstrate a similar mechanism in connection with the idiopathic variety

The evidence[4 5 7] indicates that an abnormal immunologic mechanism may be responsible for the low platelet count in many patients with this disorder Platelet agglutinins have been demonstrated in the plasma of some patients with idiopathic thrombocytopenia[7 1 8] and the platelet agglutinin has been passively transfered In some instances it is not possible to demonstrate platelet destruction hence a decrease in the production of platelets may be responsible for the thrombocytopenia[1 3] The major role played by the spleen is to remove the sensitized platelets but it may also contribute to the production of platelet agglutinins and in some manner suppress megakaryocyte function in the bone marrow[50]

CLINICAL EXAMINATION The disease may begin insidiously with an increased tendency to bruise followed in days or weeks by the appearance

acid deficiencies may result in thrombocytopenia Sequestration of platelets in *hemangioendothelioma*[46, 6] *may render them susceptible to destruction* During hypothermia platelets have been shown to be sequestered in the spleen liver and possibly the intestinal tract and marrow The sequestered platelets have been shown to return to the circulation with rewarming[14] Increased permeability of the capillaries during heat stroke is thought responsible for a decrease in the number of platelets[1, 0]

Several different platelet types have been demonstrated[6] and thrombocytopenia may occur secondary to blood transfusions as the result of incompatibility of platelet types Stasis due to sludging or excessive utilization of platelets in those cases of incompatible transfusion accompanied by intravascular clotting may result in thrombocytopenia[6] The mechanism responsible for thrombocytopenia which may occur following massive transfusions is obscure[3] An enlarged spleen may trap large numbers of platelets The resulting stasis which renders platelets susceptible to destruction is believed responsible for the thrombocytopenia associated with splenomegaly The possibility that the spleen may produce a humoral factor which depresses megakaryocytic function also has been considered[6] Viremia and bacteremia are thought to cause agglutination and destruction of platelets in vivo[6, 68] Megakaryocytes may appear inactive and diminished in number as well Thrombocytopenic purpura may be the presenting problem of patients with disseminated lupus erythematosus but the mechanism responsible is unknown[1]

The manner in which certain drugs foods or chemicals induce thrombocytopenia in susceptible individuals has been the subject of extensive investigations[5, 14, 6, 1, 8, 1, 9] Whether increased destruction or decreased production is responsible for drug induced thrombocytopenia has been for many years the subject of a lively debate It is now known that immunologic factors can affect both the rate of destruction and production of platelets[3, 6] Together a drug and platelet act as a hapten and exhibit weakly antigenic properties Antibodies may be formed which will destroy the platelets on the next exposure to that particular drug The four factors necessary to demonstrate agglutination and lysis of platelets are (1) platelets (2) the drug (3) serum from the drug sensitive patient and (4) complement Agglutination alone may occur without complement[6] The factor responsible for the lysis of platelets is in the serum of the affected patient Lysis and complement fixation may be demonstrated in vitro using serum from an affected patient the drug and his own or normal platelets but will not occur if normal serum is used The immunologic nature of the mechanism of this form of thrombocytopenia has been amply demonstrated[6] and the sensitivity has been passively transferred[134] As the exhibition of minute amounts of the offending drug after recovery may cause severe thrombocytopenia to recur in these patients it is best not to test the drug in vivo to confirm the suspicion of sensitivity

MANAGEMENT It is clear in light of the above discussion that a searching history must be obtained from any patient presenting with thrombocyto

cytopenic purpura as being of the acute or chronic variety must be retrospective and is of little help in the handling of the new patient Death may result in either the acute or chronic type and recurrences may occur after many years of remission

TREATMENT Evaluation of the various forms of treatment used in this disorder is made difficult by its unpredictable course [6, 13] The rationale for the use of adrenocortical steroids lies in the observation that they decrease antigen antibody reactions and protect the vascular endothelium in some fashion as yet not understood Initially one of the adrenocortical steroids is administered in high doses and then gradually decreased as improvement occurs A maintenance dose is continued for several weeks and the dose is gradually decreased under careful observation Whole blood transfusions are withheld unless there is life-threatening hemorrhage Platelet transfusions may be of value immediately prior to emergency splenectomy [91, 110] It is essential that blood be freshly drawn in order to infuse sufficient numbers of platelets to be effective A number of different platelet types have been demonstrated and the survival time of transfused platelets decreases progressively with each succeeding transfusion probably as the result of the formation of isoagglutinins [1, 9] Splenectomy is probably best reserved unless a life threatening situation develops and bleeding cannot be controlled with adrenocortical steroids in adequate dosage It has been reported that splenectomy has a greater likelihood of being effective if platelet agglutinins are found The results of splenectomy in a series of 303 cases [9] were as follows

Complete remission	61%
Incomplete remission	17%
Failure	22%
Mortality	11%

Steroid therapy has now reduced the mortality accompanying splenectomy to a lower figure than the one presented

In reviewing our experience it was apparent that failure of hormonal therapy did not prejudice the results of splenectomy nor did the failure to respond to splenectomy affect the future response to ACTH or adrenocortical steroids Both ACTH and adrenocortical steroids produced similar results When relapse occurred following cessation of hormonal therapy remission was often obtained upon restarting therapy Capillary fragility often diminished following institution of steroid therapy without a significant alteration of the platelet count On the basis of these observations the following general plan of therapy seems indicated

1 When first seen these patients whether "acute" or "chronic are started on prednisone 60 to 80 mg daily by mouth Usually the dosage may be decreased over the next several days to 15 to 20 mg daily If there is no improvement in one or two weeks it is unlikely to occur with further therapy

2 Splenectomy is employed in the event of failure to control bleeding with steroids after a reasonable period of trial

3 Transfusions may be required to replace the blood lost The use

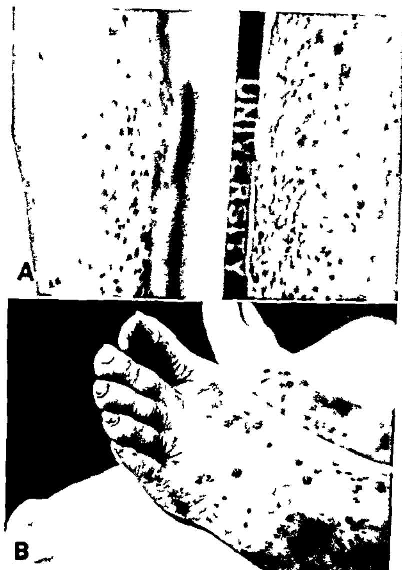


Figure 38 Petechiae and ecchymoses in a patient with idiopathic thrombocytopenic purpura

of petechiae on the skin or mucous membranes In other patients there may be a sudden onset with the appearance of petechiae and moderate to severe bleeding from the gastrointestinal or genitourinary tract Neurologic defects or death may be the result if bleeding occurs in the central nervous system The historical account of the illness and the results of the physical examination will depend largely on the site and extent of the bleeding

COURSE AND PROGNOSIS The disease may pursue an unremitting and fulminant course to death in a few days or stop as suddenly as it began within days or weeks of the onset The designation of idiopathic thrombo

The most commonly involved organs are the heart, adrenals kidneys pancreas and brain The occlusive material is acidophilic amorphous and hyalin like[47 137] Many believe that it is composed of platelets but its exact nature is unknown The thromboses may result in aneurysmal dilatation of the vessel and subsequent rupture

The hemolytic anemia which occurs in patients with this disorder is of an extracorpuscular type A fluctuation in the intensity of the hemolytic mechanism is considered responsible for the intermittent spherocytosis occasionally observed An erythrocyte which has assumed a spherical shape is considered to be in a prehemolytic state The Coombs antiglobulin test is usually negative

The thrombocytopenic manifestations of the disorder are characteristic but the mechanism by which the platelets are reduced has been the source of much debate[44 4] A shortened survival time of normal platelets in these patients has been described while infusions of plasma from patients with thrombotic thrombocytopenic purpura did not diminish the number of platelets in a normal recipient No platelet agglutinins have yet been described There is evidence of alteration in megakaryocytic function in some patients the megakaryocytes being immature

The neurologic lesions are secondary to vascular lesions in the brain

CLINICAL ASPECTS While there have been chronic or relapsing forms noted in the literature most patients present with a dramatic and fulminating disease resulting in death in a few days or weeks[44] The diagnosis is suspected in a young person critically ill with hemolytic anemia, thrombocytopenic purpura, and changing neurologic signs Positive diagnosis has been made following the finding of characteristic lesions in small vessels removed by marrow aspiration and sectioned in paraffin block Muscle biopsy may also be helpful[8]

TREATMENT Treatment is supportive No striking results have been noted from the use of steroids splenectomy or antibiotics Many feel however that steroids have not had wide enough use for final judgment to be made Steroids are used in hope that they may interfere with the underlying mechanism producing the disease Their effectiveness in other forms of hemolytic disease justifies their use in these cases Transfusion should be used to replace blood loss from hemorrhage or hemolysis

Illustrative Cases

Secondary Thrombocytopenic Purpura A 14 year old white girl had been well until three or four days prior to admission when she contracted measles which was epidemic in her community and present in her home Eighteen hours before admission she had a sudden epistaxis and later vomited blood and passed tarry stools Her mother who had raised fourteen children and knew the measles" stated that she had noted a change in the character of the rash during the 24 hours prior to admission

of platelet transfusions is limited because of the rapid destruction of platelets

4 If remission obtained with steroid therapy is followed by relapse the patient may be given another course If failure recurs splenectomy is carried out

It is important to note that idiopathic thrombocytopenic purpura may recur weeks months or even years after splenectomy The question as to just when a splenectomy should be performed must be answered for each patient individually depending on the course of disease

(c) **Neonatal Thrombocytopenic Purpura** Thrombocytopenic purpura occurring at the time of delivery may affect both the mother and baby the baby alone or the mother alone [6, 7, 141] The infant usually appears normal at the time of delivery but develops purpura several hours later The hemorrhagic manifestations may disappear after a few days but the thrombocytopenia often persists for weeks or months

MECHANISMS Investigators have ascribed the pathogenesis of neonatal thrombocytopenic purpura to the same mechanisms as those shown to be operative in idiopathic thrombocytopenic purpura [7] The mother may have autoagglutinins (even after splenectomy) which cross the placental barrier and sensitize the platelets of the child [141] The platelets which have been sensitized by the agglutinins received from the mother will be destroyed in the presence of the child s spleen After delivery the agglutinin titer will fall and the thrombocytes will rise to normal levels A second mechanism has been likened to that of Rh sensitization Should the mother and fetus have different platelet types maternal isoagglutinins may form cross the placenta, and destroy the platelets of the fetus Soon after birth the titer falls and recovery ensues Finally the mother may have a thrombocytopenic purpura without an agglutinin and may have a normal baby

THERAPY These situations are self limiting and the aim of therapy is to tide the infant over the days or weeks until recovery occurs Steroid therapy may be very helpful Splenectomy is not indicated unless there is a failure to control symptoms with steroids and the situation is life threatening

(d) **Thrombotic Thrombocytopenic Purpura** Thrombotic thrombocytopenic purpura is an exotic malady first described by Moschowitz in 1925 [90] The syndrome is now being recognized more frequently though less than one hundred cases have been reported in the literature [1, 14] It occurs most commonly in the female in the 10 to 40 age group and is marked by the striking triad of hemolytic anemia thrombocytopenic purpura and changing neurologic signs

MECHANISM The relationship of this disorder to the collagen diseases was pointed out quite early and the suggestion that it is primarily the result of a vascular lesion has recently received support [44, 47] The characteristic pathologic lesion of thrombotic thrombocytopenic purpura is partial or complete occlusion of many small arterioles and capillaries (rarely venules) [47] These lesions are widespread in the body and any organ may be involved

blood The bleeding time was 12 minutes tourniquet test positive and clot retraction poor after two hours There were no platelets seen on examination of multiple smears The prothrombin time and coagulation time were normal Preparations made to demonstrate the lupus erythematosus cell phenomena were negative on three occasions Bone marrow megakaryocytes were diminished in number and immature in appearance Atypical lymphocytes and young plasma cells were seen on examination of the bone marrow smear and the smear of the peripheral blood A diagnosis of thrombocytopenia secondary to measles was made

COURSE. Because of continued bleeding and low hematocrit the patient was transfused with 500 ml of whole blood Twenty five mg of ACTH was given intravenously over a 6 hour period, and prednisone was administered orally in a dosage of 25 mg three times a day The patient s nose was packed and bleeding was controlled adequately though there was slight oozing for the first four days Platelets which were seen for the first time on a smear made on the sixth hospital day numbered 216 000 by the eleventh hospital day At that time the bleeding time had returned to 2½ minutes The patient was discharged to be followed in the Hematology Clinic Instructions were given to reduce the prednisone gradually Her platelets remained at normal levels for the four months she was followed after steroid therapy had been discontinued

COMMENTS This girl presents the classic clinical and laboratory findings of thrombocytopenic purpura In addition to the failure to find platelets in the peripheral blood the megakaryocytes which were seen in the marrow preparation were immature and did not seem to be forming platelets L E preparations were obtained because purpuric lesions may be the presenting complaint of patients who are subsequently found to have disseminated lupus erythematosus The presence of the atypical lymphocytes and young forms of plasma cells suggested the presence of a virus disease In view of the history of measles it was felt that this represented *secondary thrombocytopenic* purpura occurring as the result of measles

Thrombocytopenia is a rare sequela of measles The appearance of the purpuric rash has been reported [7] as occurring from the third day of the measles rash to as long as two weeks subsequent to its disappearance Contrary to popular opinion purpura does not follow only severe attacks of measles and the accompanying infection may be but a mild one

The use of blood transfusion was simply for blood replacement Steroids were used to sustain the patient through what proved to be self limited acute thrombocytopenia secondary to an infectious viral disease

The prognosis is good in this case and it is doubtful if she will have a recurrence However the patient was instructed to return immediately if symptoms recurred

Idiopathic Thrombocytopenic Purpura HISTORY A 24 year old white married mother had been in good health until four months prior to admission when she noted bruising without recognized trauma Bruising continued and when she sought medical attention three months prior to admission she was told that her platelets were less than 10 000" Intramuscular injections

Careful questioning failed to reveal any history of a hemorrhagic diathesis in the patient or in her family There had been no known exposure to chemicals and she had received no drugs

PHYSICAL EXAMINATION There were many petechiae over the lower legs and back and subconjunctival hemorrhages were noted in both eyes There was a small amount of blood in the posterior pharynx and mild oozing from the nares There were small nodes in both the posterior cervical triangles and the suboccipital area but the spleen was not palpable The remainder of the examination was non contributory

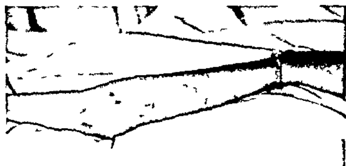


Figure 39 Positive tourniquet test in a patient with secondary thrombocytopenic purpura



Figure 40 Pathologic lymphocyte in peripheral blood of patient with secondary thrombocytopenic purpura following measles

LABORATORY EXAMINATION Laboratory examination revealed a hematocrit of 26 per cent and a white blood cell count of 11 000 per cu mm Urinalysis was negative but the stool examination was positive for occult

blood. The bleeding time was 12 minutes tourniquet test positive and clot retraction poor after two hours There were no platelets seen on examination of multiple smears The prothrombin time and coagulation time were normal. Preparations made to demonstrate the lupus erythematosus cell phenomena were negative on three occasions *Bone marrow megakaryocytes* were diminished in number and immature in appearance Atypical lymphocytes and young plasma cells were seen on examination of the bone marrow smear and the smear of the peripheral blood A diagnosis of thrombocytopenia secondary to measles was made

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of ACTH gel were given with improvement The platelets returned to normal levels and bruising ceased After an interval of two or three weeks excessive bruising was again noted and upon examination platelets once more were found to be low With the onset of excessive menstrual bleeding she was referred for further treatment No significant family or personal history could be elicited There had been no exposure to toxins nor had there been any recent infections

PHYSICAL EXAMINATION The physical examination revealed many ecchymoses over the upper and lower extremities Several petechiae were noted on the buccal mucosa The remainder of the examination was negative No lymph nodes were enlarged and the spleen was not palpable

LABORATORY EXAMINATIONS Laboratory examinations revealed a hematocrit of 41 per cent and a white blood cell count of 10 300 per cu mm Differential white count was normal Urinalysis was normal and examination of the feces was negative for occult blood The platelet count was 22 000 the bleeding time 18 minutes and the tourniquet test positive The coagulation time and prothrombin time were both normal A diagnosis of idiopathic thrombocytopenic purpura was made

COURSE Since the patient had responded once to adrenocortical steroid therapy it was decided to attempt once again to control her bleeding with these compounds Accordingly oral prednisone 15 mg every 6 hours (60 mg a day) was begun After four days the platelet count was 150 000 per cu mm and bruising had ceased The dose of prednisone was reduced to 10 mg every 6 hours (40 mg a day) and improvement continued On the sixth day the platelet count was 409 000 per cu mm and the dosage of prednisone was reduced to 5 mg four times a day The patient was discharged in the care of her physician and was instructed to take 5 mg prednisone three times a day

The dosage of prednisone was decreased gradually and after two months it was discontinued Immediately her platelets fell to less than 10 000 In view of the two relapses following withdrawal of steroid therapy it was decided that splenectomy should be performed Accordingly prednisone was restarted in preparation for operation and continued through the postoperative period The platelet count at time of operation was 748 000 per cu mm and on the first postoperative day was 898 000 The patient was discharged with instruction to reduce the prednisone gradually over an eight day period She continued well during the following year without the support of adrenocortical steroid therapy

COMMENT Careful search failed to reveal any primary etiology and a diagnosis of idiopathic thrombocytopenic purpura was made Laboratory findings confirmed the diagnosis in that bleeding time and tourniquet test were both abnormal and the finding of a low platelet count revealed the reason for the abnormal tests

Splenectomy was performed because adrenocortical steroid administration could not be discontinued without relapse after a six month trial period The increase in thrombocytes after splenectomy is but one of the charac

teristic changes in the peripheral blood after this operation Others include the appearance of target cells Howell Jolly bodies leukocytosis and nucleated red blood cells These changes have been thought to indicate that the spleen exerts some inhibitory effect on the bone marrow Another explanation may be that removal of the spleen decreases the peripheral destruction of these particular elements A relapse may occur in this patient as long as 20 to 25 years after splenectomy Should this occur a decision must then be made as to whether she may have developed an accessory spleen and warrants re exploration

NON THROMBOCYTOPENIC PURPURA

Patients with non thrombocytopenic purpura may have all of the hemorrhagic lesions noted for those with thrombocytopenic purpura They differ in that their platelet count remains within the normal range Characteristic lesions include petechiae ecchymoses hematoma or spontaneous bleeding from the genitourinary tract or gastrointestinal tract The hemorrhagic lesions may be present in the skin or mucous membranes

(a) *Allergic Purpura*

This disorder known also as the Schoenlein Henoch syndrome was extensively studied by Osler[97] and his reports are the basis of much of our present day knowledge of the variability of the clinical findings

Clinical Examination The presenting complaints will depend on the site and extent of the involvement Osler[97] remarked that the two outstanding clinical features were recurrence at long or short intervals and marked variability of the skin lesions The syndrome characteristically recurs several times before final remission The recurrences may appear over a period of weeks months or in some cases years The underlying pathology allows the development of skin lesions of great variability There may be only purpura with petechiae and ecchymoses or there may be associated effusion erythema or necrosis of the skin Gastrointestinal involvement is marked by colicky abdominal pain which is often of great severity and occurs chiefly at night Diarrhea and vomiting are frequent and both the vomitus and stools may contain blood[3 97] Hematuria is also a frequent finding and nephritis commonly accompanies the disorder[3 33 10] Joint symptoms may be the presenting complaint Several joints are usually involved by pain at one time and there may or may not be associated swelling

The prognosis for life is generally good unless there is involvement of some unfortunate site such as the glottis The disease is marked by repeated attacks which may extend over months or years though most recover after a month or so

There is no consistent laboratory finding There may be an anemia if the bleeding has been extensive enough but this is rare Platelets of course

are normal or only slightly reduced The capillary fragility test is positive in only a portion of the cases[3]

Mechanism The etiology of this disorder remains unknown There is increased capillary permeability which allows the escape of elements of the blood into the surrounding tissue but the nature of the capillary lesion is not clear In some cases infection may play a role Streptococcal infection of the throat has been implicated by some[10] but the evidence is weak[3] Hypersensitivity to certain foods has been implicated in some instances of this disorder and recovery has followed the avoidance of the offending agent Some have pointed to a resemblance between the lesions of allergic purpura and those of the collagen diseases but again the evidence is incomplete[33]

Treatment The treatment of this syndrome is largely supportive A careful search should be made for an offending food in the chronic cases Throat culture should be made and dealt with as indicated The frequency with which the syndrome is accompanied by nephritis makes it imperative to pay close attention to the cultures Ackroyd feels the patients should be kept in bed in the hope of reducing the incidence and severity of nephritis[3] Steroid therapy has not had wide trial but the results have been disappointing thus far[133]

(b) Infectious Purpura

Purpura in association with infection has been known since ancient times[67] It is a recognized and expected clinical finding in such disorders as Rocky Mountain spotted fever typhus meningococcemia and bacterial endocarditis In each of these disorders an escape of blood elements into the surrounding tissue follows damage to the vascular endothelium

There are instances in which purpura is associated with severe forms of exanthems such as chickenpox or smallpox In these instances the purpura seems to occur because of the unusual severity of the disease which causes damage to the vascular endothelium[68]

Finally purpura may be associated with infection in certain patients who exhibit what appears to be individual susceptibility The purpura may be thrombocytopenic or non thrombocytopenic Both types have occurred in association with scarlet fever varicella measles tuberculosis and infectious hepatitis[3] These diseases need not be particularly severe for purpura to be a complication The natural history of the purpura in these instances is similar to that of idiopathic thrombocytopenic purpura

The mechanism responsible for the purpura is not clear No histologic change in the vascular system has yet been demonstrated

TREATMENT While treatment is directed to the underlying disease with the use of appropriate antibiotics care should be taken to tide the patient over the hemorrhagic episode Adrenocortical steroids may be helpful but caution must be observed in light of recent reports of the extraordinary severity and frequent deaths from varicella in children who have been on steroid therapy[6] Adrenocortical steroids should be used with caution in

varicella if at all and should be used with caution in other virus exanthemas Splenectomy would not be indicated except possibly in chronic cases for example those associated with tuberculosis involving the spleen

(c) Other Types

Non thrombocytopenic purpura may occur following exposure to chemicals or drugs to which the individual is susceptible[3] in vitamin C deficiency[53] as the result of certain snake bites[9] and in disorders characterized by the presence of abnormal serum proteins In the last case deposition of abnormal proteins in the small vessels alters their permeability and allows escape of



Figure 41 Senile purpura

fluid content This is seen in multiple myeloma in association with cryo globulins or para amyloid Waldenstrom has described[144] a macroglobulinemia usually occurring in elderly men and characterized by the presence of abnormal lymphocytes in the bone marrow and a globulin of high molecular weight in the serum In this disorder purpura occurs particularly over the lower extremities Purpura may also occur in association with cold agglutinins for example following virus pneumonia Non thrombocytopenic purpura may be a feature of old age presumably as the result of loss of support to the small vessels

HEREDITARY HEMORRHAGIC TELANGIECTASIA

This hereditary vascular anomaly was first described in 1896 by Rendu[98] In 1901 Osler[96] reported several cases in detail and noted most of the clinical

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Figure 42 Hereditary telangiectasia involving (A) tongue and (B) lips

findings known today Weber[146] reported an extensive family study in 1907 and two years later Hanes [1] made further contributions on the subject and suggested the name by which the disorder is known today

Pathologic Physiology Hereditary hemorrhagic telangiectasia is genetically transmitted by either sex as an autosomal dominant Often the disease may not become apparent until the second or third decade when the lesions develop and gradually increase in number Abnormal bleeding may be delayed until middle life and often grows more severe with age Some patients are unaffected by the presence of telangiectasia and hemorrhage does not occur These lesions are composed of greatly dilated thin walled vessels which are reported [1] to have inadequate smooth muscle and elastic tissues

Clinical Examination The characteristic vascular lesions are pin point in size or several millimeters in diameter They are red or purple in color and slightly raised from the surrounding surface They may blanch slightly with pressure The lesions which may occur anywhere on the surface of the body or in any organ are most frequently found on the skin of the face the ears or the hands and feet or on the mucous membranes of the nose and mouth

The diagnosis must be suspected in cases of recurrent hemorrhage associated with telangiectasia and a family history of abnormal bleeding The most common complaints are those of hemorrhage and anemia Hemorrhage may occur as the result of slight trauma to one of the vascular lesions anywhere on the body or may be spontaneous Hemorrhage from the nose or mouth is common but bleeding may occur from the respiratory tract gastrointestinal tract or genitourinary tract as well

The chief laboratory findings are those of anemia There may be iron

deficiency with low serum iron and high iron binding capacity if bleeding has been chronic Coagulation studies are completely normal

Treatment The use of electrocoagulation has been successful in controlling some of the accessible lesions One of Osler's patients used a small balloon which he inserted into his nose in a deflated condition and then inflated to control nasal hemorrhage This procedure is used with success today The management of hemorrhage from the lesions of hereditary hemorrhagic telangiectasia presents a difficult problem when they are located in the gastrointestinal tract They are difficult if not impossible to localize by x ray but occasionally may be demonstrated by endoscopy [148] While resection may remove one source of hemorrhage the future development of similar lesions remains a constant threat

II ABNORMALITIES OF BLOOD COAGULATION

DISORDERS AFFECTING PREDOMINANTLY THE FIRST STAGE OF BLOOD COAGULATION

The following disorders are grouped together because of the similarity of their clinical and laboratory findings Each results in a delay in the development of blood thromboplastin activity to a greater or less extent [3 1 3] but produces no alteration in the one stage prothrombin time

Hemophilia is one of the oldest recognized bleeding disorders and affected members of several of the ruling houses in Europe It has been recognized in recent years that the clinical findings of classic "hemophilia" may result from any one of three distinct genetic defects or from acquired circulating anticoagulants These disorders can be differentiated only by appropriate laboratory tests since they present similar clinical findings It is for this reason that accurate classification of the cases presented in the older literature as hemophilia" is not possible The three genetic disorders now recognized with their relative incidence in two series [41 11 113] are as follows

1 Antihemophilic factor (AHF) deficiency 80–82%
2 Plasma thromboplastin component (PTC) deficiency 11–15%
3 Plasma thromboplastin antecedent (PTA) deficiency 5–7%

1 *Antihemophilic Factor (AHF) Deficiency*

Pathologic Physiology Antihemophilic factor (AHF) deficiency is passed from one generation to the next as a sex linked recessive Ordinarily the female is a carrier and manifests no clinical signs or symptoms of the disorder

The genetic possibilities inherent in this and similar disorders are best indicated by the use of symbols and diagrams By common usage the male is designated as XY while the female is designated XX In order for a male to be conceived, the male parent must contribute the Y chromosome while

the female contributes the X Specific genes are usually referred to by super script letters A dominant gene is designated by a capital letter and a reces sive by a lower case letter

The abnormal gene responsible for AHF deficiency is recessive and is designated by the lower case letter h The normal gene which is dominant is designated by a capital H A normal female would thus be designated X^HX^H The carrier female would be X^HX^h In the case of the carrier female the dominant normal gene suppresses any expression of the recessive abnormal gene The carrier has no symptoms The Y chromosome of the male does not carry this gene and has neither H nor h Whether a male child has AHF de ficiency or not depends entirely on the character of the X chromosome he must receive from the female If the female carrier contributes the normal X^H to the male offspring the male is normal If however the X chromosome contributed by the female to her male offspring is that which carries the abnormal gene it would find expression as there would be no normal gene (H) to suppress it

The following diagrams will illustrate the possibilities The male in each instance is designated by the rectangle and the female by the circle

Figure 43 represents a normal family with no factor deficiency All off spring are normal

Figure 44 indicates that a mating of a normal male and a carrier female may result in a hemophilic male or a carrier female as well as normal chil dren of both sexes Unfortunately there is no way to establish with certainty whether a female is a carrier or not There is evidence however that the degree of recessiveness may vary and that some carrier females may have depressed AHF levels

Figure 45 indicates the results of a mating of an AHF deficient male with a normal female All of their female children *must* be carriers since the only

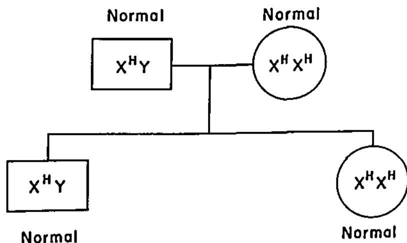


Figure 43

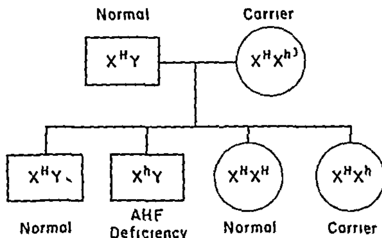


Figure 44

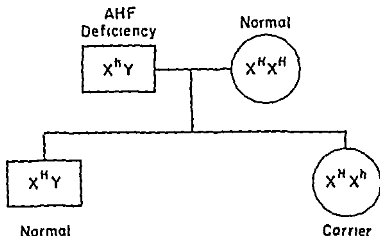


Figure 45

X chromosome the male has to contribute carries the abnormal gene On the other hand none of the male offspring can have hemophilia since a male must receive the lone X chromosome from the normal mother

In Figure 46 we see the results of the mating of an AHF deficient male and a carrier female

For many years the possibility that a female could have an AHF deficiency was not accepted but several well documented cases have now been reported.[86] The female offspring of the mating noted here would receive an X^h from the AHF deficient male and the second X^h from the carrier mother

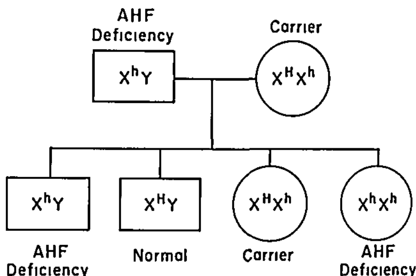


Figure 46

In Figure 47 we see the result of a mating between a normal male and an AHF deficient female

All male progeny would receive X^h from the mother while all female would receive an X^H from the father in addition to the X^h from the mother All males would then be AHF deficient and all females would be carriers

The mating of a male and a female both deficient in AHF has probably never occurred except in laboratory animals All of the offspring of such a mating would be AHF deficient (Fig 48)

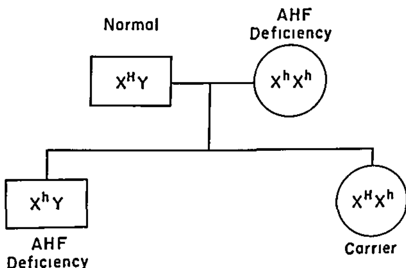


Figure 47

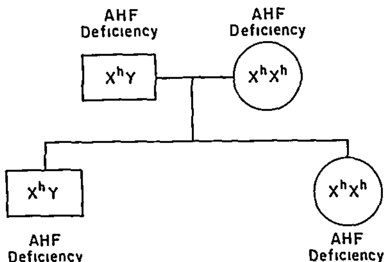


Figure 48

As there are patients with hemophilia who have no family history of the disorder the rate of mutation is thought to be high

The result of the genetic defect is known to be the deficiency of a globulin first described by Patek and Taylor[100] In the absence of this globulin the first phase of coagulation is impeded Since the platelets and the capillaries are unaffected, the bleeding time is normal while the coagulation time is often prolonged The more severe the deficiency of AHF the more severe the bleeding problem Levels of below 3 per cent are usually found in symptomatic patients though a bleeding diathesis may be found in patients with levels as high as 20 per cent The level of AHF in any one patient is remarkably constant throughout his life

AHF is one of the factors needed in the development of blood thromboplastin activity and in its complete absence the only effective hemostatic defense left to the patient is that supplied by the vascular phase of hemostasis and the extrinsic system In the event of a defect in the larger vessels these defenses are not sufficient to maintain hemostasis and extensive bleeding may occur

Clinical Aspects In the past many born with AHF deficiency died in infancy or in the first five years but with modern therapy there has been marked improvement in the prognosis The availability of adequate transfusion service has probably been the most important factor in the reduction of mortality from this disorder

Mildly affected hemophilics may reach adult life before serious bleeding occurs The more severely affected individuals may have repeated episodes of bleeding from the surface of the body the serous surfaces the gastrointestinal tract the genitourinary tract or into joint spaces Bleeding does

not often if ever occur in the absence of trauma The hemarthroses which are suffered often result in permanent ankylosis and deformity

Elective surgery is denied these patients and dental extractions must be done with great skill and careful preparation by dental surgeons aware of the problem *Until recently conditions which made surgery imperative were* catastrophic Now it is possible to prepare for such emergencies with a reasonable chance of success [37, 84]

For many years the diagnosis of "hemophilia was made when it could be demonstrated in vitro that plasma from the patient in question did not correct clotting defect of a patient with known hemophilia The diagnostic difficulties previously encountered are now recognized to have occurred because any one of several defects can produce clinical findings of classic *hemophilia These defects may be identified only by proper laboratory tests* The platelet count tourniquet test and bleeding time are unaffected as they depend on capillary integrity and platelets both of which are normal in AHF deficiency Clot retraction is likewise normal since there is adequate fibrin and normal platelet function The prothrombin time is normal since *this test system bypasses the factor deficient in hemophilia*

The coagulation time may be prolonged owing to a decrease in the formation of thromboplastin activity It is important to note however that the coagulation time may be normal despite relatively severe AHF deficiency The thromboplastin generation test can be used to identify the specific defect in any of these disorders [19] Modifications may be made in the thromboplastin generation test which enable one to determine the percentage of AHF activity present in the suspect plasma

Treatment The treatment of patients with hemophilia has improved considerably in recent years Modern blood banking methods have made available large quantities of whole blood and plasma high potency preparations of AHF factor from animal blood have recently become available[61] and the use of concentrates of human AHF factor is now under trial [69] While *none of these materials provide the answer" for hemophilia as does vitamin* B_{12} for pernicious anemia they have nonetheless proved a great boon to the hemophilic Blood loss is more quickly replaced and hemostasis secured If the patient is properly prepared tooth extraction so often denied these patients in the past despite desperate need is now reasonably safe and major surgery though still extremely hazardous is at least feasible

The use of human plasma as a source of AHF has been successful in controlling most hemorrhage into soft tissues hematuria, and the bleeding which *follows tooth extraction in those hemophilic patients with a mild deficiency* It must be freshly prepared for it loses over 50 per cent of its activity in four days if stored at usual blood bank temperatures of 2 to 4° C [69] Plasma which has been quickly separated and frozen at − 30° C retains its activity for as long as 30 days however and is an excellent product to have available *Unfortunately* hemostatic levels of AHF cannot be obtained in the severe hemophilic using plasma alone The AHF level of human plasma is rela

tively low and the circulatory system would be overloaded before an adequate concentration could be reached

The need for a source of concentrated AHF has long been recognized Successful preparation of high potency material from animal blood[17, 81] has made it possible to give to the patient an amount of AHF equivalent to that found in 8 liters of human blood in a single infusion of small volume However as these preparations are of heterologous protein they are antigenic and are thus limited in their usefulness It has proved possible to give a single course of therapy to a patient over eight to ten days but the response to the infusions often progressively diminishes A second course is prohibited by the possibility of anaphylaxis Since there are only two animal preparations available (bovine and porcine) they are reserved for dire emergencies

The preparation of concentrates of human AHF holds considerable promise With these preparations an amount of AHF equivalent to that found in a liter of plasma can be given in 100 ml Its use is still in the experimental phase and its antigenicity has not yet been determined[69]

Dental extractions have been rendered more safe by the use of dental splints of acrylic resin[149] These splints protect the gum and prevent the dislodgment of a clot once formed Calcium alginate gauze soaked in Russell viper venom may be placed between the splint and the gum to act as a local hemostatic agent A liter of fresh plasma is given these patients an hour before the extraction and thereafter as indicated Under no circumstances should the gum margins be sutured as bleeding may continue beneath the sutures and dissect into the fascial planes of the neck causing respiratory obstruction

Hemorrhage in the tissue of the throat, at the base of the tongue or in the neck is usually controlled by the infusion of fresh plasma The possibility of respiratory obstruction should be appreciated in time to enable an endotracheal tube to be passed Tracheotomy should be avoided if possible as it increases the danger of serious hemorrhage The blood in these tissues does not localize and the surgeon should not attempt drainage as such efforts result only in further bleeding and increased danger to the patient The hemorrhage will cease and the blood will be absorbed quickly if an adequate concentration of AHF is obtained Superficial hemorrhage is controlled by the immediate application of local pressure while the infusion of fresh plasma is started After an elevation of the AHF is obtained the pressure should be released to allow blood with adequate AHF to replace the AHF deficient blood in the blocked vessels and then reapplied

Hemarthroses should be treated as conservatively as possible Bed rest and ice bags to the affected joint during the acute phase with cautious activity as improvement occurs seem indicated Repeated aspirations and lavage may lead to infection or further bleeding and should be avoided if possible[131] Plasma should be given to raise the AHF level It is desirable to have the joint in the most functional position should ankylosis occur

Operations should be avoided if possible Should major surgery be essential a concentration of not under 25 per cent AHF must be obtained Since

this is not possible using plasma alone animal or human AHF must be available It is essential that surgery on these patients be carried out in centers where careful laboratory control can be exercised[37, 84]

Illustrative Case HISTORY An 18 year old white male was admitted for the first time because of continued bleeding for six days from the site of a tooth extraction This young man stated that he had been told by his mother that he had been a "bleeder" since birth He had always bled easily from minor cuts and abrasions bruised quite easily and had frequent nose bleeds He had suffered bluish" swelling of the right knee and painful hip joints Because of his bleeding tendency he had never been allowed to go to school Six days prior to admission he had had a right lower molar extracted The gum flaps were sutured as bleeding continued after the extraction Several hours later a mass was noted at the angle of his jaw and bleeding from the gum continued He was seen by his physician who referred him for treatment

Family history revealed that his mother and father were cousins One brother was known to have a bleeding dyscrasia Two sisters and the other brother were normal

PHYSICAL EXAMINATION The patient was a thin pale young man lying quietly in bed. Examination revealed a large clot in his mouth overlying the right gum from beneath which blood was still oozing An indurated 3 × 4 cm mass was palpable below the angle of the right ramus of the mandible A large ecchymosis of the skin extended from the area occupied by the mass to the interior chest wall on the right There was no lymphadenopathy and no organs or masses were palpable in the abdomen

LABORATORY EXAMINATION Laboratory examination revealed a hematocrit of 28 per cent and white blood cell count of 5600 per cu mm Platelet count bleeding time and tourniquet test were normal The coagulation time was 16 minutes and the prothrombin time normal Thromboplastin generation test was abnormal when the patient s plasma was substituted for normal plasma in the incubation mixture The patient s serum was normal Assay revealed less than 1 per cent AHF activity No circulating anticoagulants could be demonstrated

Subchondral cystic change and irregularity of articulating surfaces of both hips and knees were noted by x ray These changes were felt to be consistent with hemophilic arthritis A diagnosis of antihemophilic factor deficiency was made

COURSE The patient was transfused with 2000 ml of fresh whole blood during the next 48 hours The bleeding ceased and the clotting time returned to 11 minutes A dentist removed the sutures from the gum flap and cleaned the area gently after which a firm clot formed

COMMENT This case history demonstrates several features of importance It is a classic example of the grave risks which are encountered when tooth extraction is attempted without proper preparation The risks were compounded by the suturing of the gum flaps This lad is fortunate to have

escaped asphyxiation which might have resulted from continued bleeding *interiorly*—beneath the sutured flaps with dissection of *fascial planes of the* neck

The use of fresh whole blood (or plasma) to control bleeding of this sort is demonstrated to be of value The amount of blood given would not have been expected to raise the AHF concentration greatly but was adequate It *should* be noted that the coagulation time returned to normal despite the fact that the AHF concentration was still very low This test may not reveal coagulation defects *if they are not severe*

The social problem presented by this patient is quite apparent His parents had been advised never to send him to school He had had no education whatsoever and without his parents would be unable to support himself These children should be protected from trauma but they must not be denied an education

The use of the thromboplastin generation test clearly separated this AHF *deficiency from the other defects in the first phase of* coagulation

The patient s brother was subsequently brought to the hospital and was demonstrated to have AHF deficiency

2 *Plasma Thromboplastin Component (PTC) Deficiency*

The practice of comparing the coagulation characteristics of plasma from patients with undiagnosed hemorrhagic disease with those of plasma from clinically accepted cases of hemophilia in order to establish the diagnosis of *hemophilia has in the past led to considerable confusion* [1] [3] *In* 1947 Pavlosky[101] reported that the addition of blood from one hemophilic patient to that of another *in vitro* occasionally resulted *in a normal coagulation time* Aggeler et al [7] and Biggs et al [18] later recognized that the existence of a heretofore unrecognized factor must be postulated and that the absence of this factor would produce a syndrome clinically indistinguishable from hemophilia This factor most commonly known as the plasma thromboplastin component (PTC) or the Christmas factor has been extensively investigated Many patients thought to have AHF deficiency have now been recognized to have a deficiency of this factor

Pathologic Physiology This defect is transmitted as a sex linked recessive *with genetic possibilities similar to those described* above under AHF factor deficiency The gene is not as recessive as that responsible for AHF deficiency and symptoms may occur in female heterozygotes

Like the AHF plasma thromboplastin component takes part in the first phase of the coagulation of blood A deficiency of this factor impedes the development of thromboplastin activity The platelets and capillaries are unaffected and repair of small capillary rents proceeds normally

Clinical Findings These patients may present any *of the problems* found in AHF deficiency Males are more commonly affected though PTC deficient females *are not as uncommon as those with* AHF deficiency This

is perhaps a reflection of the fact that this gene is less completely recessive than that responsible for AHF deficiency and is finding expression in hetero zygotes Manifestations of the hemorrhagic diathesis may appear at birth with bleeding from the umbilical cord or following circumcision Excessive bleeding will mark minor trauma and surgical procedures throughout the life of the patient.

The laboratory findings in this disorder follow the same general pattern as those in AHF deficiency The platelet count capillary fragility clot re traction bleeding time and prothrombin time are normal

The coagulation time is often prolonged since the development of throm boplastin activity is impeded As in AHF deficiency however PTC deficient patients may have normal coagulation times

As PTC is not consumed during coagulation it is present in normal serum As a consequence the coagulation of PTC deficient plasma may be brought to normal in vitro by the addition of normal serum The defect will not be corrected by the addition of $BaSO_4$ treated normal serum or plasma since $BaSO_4$ adsorbs PTC completely This disorder may be identified by the thromboplastin generation test which is based on these facts Substitution in the incubation mixture of the patient s serum for the normal serum will prevent the normal evolution of thromboplastin activity

Treatment The treatment of this disorder is perhaps more difficult than that of AHF deficiency as there are at present no concentrates of PTC available and these patients may have a clinical course equally severe It is thought that PTC is stable on storage at blood bank temperatures for two or three weeks [4, 113] It has been reported that when acid citrate dextrose plasma stored at 4° C is the source of PTC the results of the thromboplastin generation test are abnormal within fourteen days and normal during the first four days of storage only However plasma stored at 4° C for fifteen days and longer has been reported to raise the PTC level of patients with known PTC factor deficiency [4] Serum which can be shown to be a good source of PTC in vitro has been demonstrated to be ineffective in the treat ment of PTC deficiency The reason for this is not clear [94] The comments made above on the clinical management of AHF deficiency apply equally to the management of this disorder

3 *Plasma Thromboplastin Antecedent (PTA) Deficiency*

Plasma thromboplastin antecedent (PTA) deficiency was described by Rosenthal in 1953 [114] The clinical findings are similar to those of AHF and PTC deficiency but the hemostatic defect is usually less severe

Pathologic Physiology PTA deficiency is genetically transmitted as an autosomal dominant Reception of the abnormal gene from either parent will produce the disease The disorder may occur in either sex as the gene is not carried on the sex chromosome The patient will have a 50 per cent chance of passing the disorder to his or her offspring The gene has a high degree of penetrance and variable expression This indicates that the gene

will more than likely manifest itself if present but that the seriousness of the defect will vary[114 115]

PTA deficiency like PTC or AHF deficiency interferes with the development of adequate thromboplastin activity during the first phase of coagulation

Clinical Aspects Patients of either sex present with a bleeding diathesis similar to AHF and PTC deficiency The bleeding episodes are neither so frequent nor so severe as those encountered in patients with AHF and PTC deficiency and there appear to be periods when bleeding does not occur despite major trauma

The laboratory findings follow the same general pattern as that described for AHF or PTC deficiency The platelet count capillary fragility test bleeding time clot retraction and fibrinogen assay are all normal The coagulation time may reflect the impaired thromboplastin activity

Unlike AHF PTA is not consumed during coagulation and with PTC is present in both plasma and serum PTA however is not adsorbed from normal plasma by $BaSO_4$ while PTC is adsorbed Consequently the clotting time of PTA deficient plasma is corrected by $BaSO_4$ treated normal plasma which does not correct the clotting time of PTC deficient plasma It is also corrected by the addition of normal serum which would not correct the clotting time of AHF deficient plasma The three disorders may be separated by the thromboplastin generation test the design of which makes use of these facts

Treatment These patients are successfully treated with plasma stored up to fourteen days since PTA fares well on storage The effect of the administered plasma is gradually lost over a period of a week to ten days If surgery cannot be avoided these patients should be carefully prepared Prior to operation normal plasma given in amounts equal to 15 per cent of the plasma volume (about 450 ml) has been reported[115] to restore clotting time and prothrombin consumption to normal

4 *Circulating Anticoagulants*

Severe hemorrhagic symptoms may be caused by the development of circulating anticoagulants They interfere with the development of thromboplastin activity and may produce any of the findings noted to occur as the result of deficiencies of those coagulation factors concerned with the development of thromboplastin activity When circulating anticoagulants occur in patients with preexisting hemorrhagic diathesis the treatment is made far more difficult While there may be no increase in the number of hemorrhagic episodes the treatment of each episode is complicated by the need to overcome the effect of the circulating anticoagulant as well as treat the original defect Circulating anticoagulants developing during or subsequent to pregnancy may produce a severe hemorrhagic diathesis which persists for months or years[8] There have been reports of circulating anticoagulants occurring in association with a heterogeneous group of disorders including syphilis

chronic nephritis, tuberculous lymphadenopathy, and the collagen diseases.[33, 4, 9, 5] Rarely they may develop spontaneously in persons without known disease.

Clinical Findings. These patients may have any of the physical findings associated with the other disorders affecting the formation of thromboplastin activity or its function. Once again it is in the laboratory that positive identification of the disorder is made. Since the vascular phase of hemostasis is unaffected, the platelet count, bleeding time, tourniquet test, and clot retraction are normal. Fibrinogen assay is also normal as there is no interference in the final phase of coagulation. Interference by the circulating anticoagulant with the formation of thromboplastin activity results in an abnormal thromboplastin generation test. Diminished formation of thromboplastin activity occurs when the patient's serum or adsorbed plasma is substituted in the incubation mixture. The use of 50 per cent normal and 50 per cent patient's adsorbed plasma with normal serum in the incubation mixture results in diminished formation of thromboplastin activity. The presence of a circulating anticoagulant is thereby identified, as any genetic defect likely to be confused with it would have been corrected by the addition of 50 per cent normal adsorbed plasma.

Heparin-like compounds may be differentiated from circulating anticoagulants by the addition to the plasma of protamine sulfate or toluidine blue, which neutralizes the heparin-like compounds and leaves circulating anticoagulants unaffected.

Pathologic Physiology. The circulating anticoagulants have been shown to interfere with the reactions leading to the formation of thromboplastin activity in the intrinsic system.[40, 61, 6] Others have suggested they may also interfere with the action of formed thromboplastin.[3, 39, 4, 8]

They are present in the gamma fraction of the plasma and are thought to be the result of some immunologic mechanism.[9, 61, 4] The finding of circulating anticoagulants in patients treated repeatedly with blood or blood products, following pregnancy, and in association with diseases known to have deranged immunologic mechanisms has lent substance to this theory. There are, however, instances in which such a mechanism does not seem to operate and the mechanism is unclear.[7, 61] Circulating anticoagulants are relatively heat stable and fare well on storage at 0 to 4° for long periods of time. Circulating anticoagulants against AHF, PTC, Factor V and Factor VII have been described, as well as those reportedly interfering with thromboplastin activity. In those instances when circulating anticoagulants interfere with Factor V, Factor VII, or thromboplastin activity itself, the one-stage prothrombin time will of course be prolonged.

Treatment. Treatment of these patients is the same as that employed in other disorders interfering with the formation of blood thromboplastin activity. Occasionally massive transfusions of fresh blood effect a response when smaller ones do not. Adrenocortical steroids have not been used with striking success but should be tried.

DISORDERS AFFECTING BOTH THE FIRST AND SECOND STAGES OF BLOOD COAGULATION

The disorders to be discussed below affect both the first and second phases of blood coagulation The thromboplastin generation test and the one stage prothrombin time test are abnormal Both disorders may be hereditary or acquired

1 *Factor V Deficiency (Parahemophilia)*

Factor V deficiency was first demonstrated to be a distinct clinical entity by Owren[98] in 1947 The young woman whose disorder he reported suffered from epistaxis easy bruising and excessive menstrual bleeding The one stage prothrombin time was prolonged and was not shortened by the addition of prothrombin It was corrected, however by the addition of a prothrombin free plasma fraction obtained from a normal source *Thus* was it demonstrated that a deficiency of a factor other than prothrombin could not only prolong the one stage prothrombin time but could produce a serious clinical disorder as well

Pathologic Physiology Factor V deficiency may be congenital or acquired The congenital form is thought to be transmitted as an autosomal recessive although the abnormal gene has a potency roughly equal to the normal counterpart There is a high degree of variation in expressivity which is thought responsible for the finding of some heterozygotes with hemorrhagic symptoms while in others the carrier state is not detectable[71] Consanguinity has been noted to be a feature of some of the family pedigrees

The acquired form of the disorder has been reported in association with severe liver damage and a variety of other diseases[99, 1, 6] Notably the level of Factor V is depressed in association with fibrinolysis and certain instances of acquired fibrinogen deficiency As Factor V is essential to the first phase of blood coagulation its deficiency will result in the inadequate production of thromboplastin activity Fantl[36] has reported that abnormal bleeding will occur when the level of Factor V falls below 30 per cent of normal Serum does not contain Factor V because it is consumed during the process of blood coagulation

Indirect evidence points to the liver as the normal source of Factor V and acquired deficiencies have been reported in severe liver disease The factor is not vitamin K dependent and deficiency of Factor V does not occur in obstructive jaundice or during the administration of coumarin drugs

Clinical Findings In a recent review of the reported cases of congenital Factor V deficiency[36] it was noted that bleeding occurs in the first few years of life Bleeding from accidental or operative trauma was excessive and spontaneous bleeding into the genitourinary tract and the gastrointestinal tract was noted. Hemarthrosis was not common Menstrual bleeding is found to be excessive and may be life threatening

The clinical findings of the acquired form of the disorder are similar to

those of the congenital variety complicated of course by the underlying disease

The vascular phase of hemostasis is unaffected Platelets are normal in number and function and the bleeding time and tourniquet test are normal There is no quantitative or qualitative defect in fibrinogen

Since Factor V deficiency affects the first stage of coagulation it is reflected in an abnormal thromboplastin generation test The one stage prothrombin time is prolonged since Factor V is essential for the normal evolution of thrombin from prothrombin under the conditions of the test Inadequate formation of thromboplastin activity is responsible for the abnormal coagulation time

The laboratory findings in the acquired form of the disorder will of course be modified by the associated disease

Treatment Treatment consists of the administration of fresh plasma by the intravenous route Fantl[16] has reported that it is necessary in the case of complete deficiency to administer 10 ml of fresh citrated human plasma per kilogram of body weight in order to attain adequate levels The half life of the Factor V is reported to be approximately twenty hours

2 *Stuart Factor Deficiency*

In 1957 Hougie Barrow and Graham[60] restudied a patient thought to suffer from a deficiency of Factor VII Their careful work established the heterogeneity of the coagulation defects in the patients in this group A new blood clotting factor was uncovered and its properties detailed in their report

Pathologic Physiology Stuart factor deficiency has been demonstrated[18] to be transmitted by an incompletely recessive autosomal gene While most severe in the homozygote bleeding tendencies have been noted in the heterozygote and carriers can often be detected with appropriate tests

Recently Graham[4] has described a temporary deficiency of Stuart factor developed presumably as the result of contact with a farm insecticide

Stuart factor has been demonstrated to be essential in the first phase of blood coagulation and a deficiency of the factor is demonstrated by the thromboplastin generation test The defect is apparent when the patient's serum is substituted for normal serum in the incubation mixture Stuart factor is adsorbed by $BaSO_4$ from normal plasma As $BaSO_4$ treated plasma is used in performance of the test the defect is not detectable when the patient's $BaSO_4$ treated plasma is substituted for normal Both normal and patient plasma are deficient in Stuart factor under conditions of the test Stuart factor is present in normal serum as it is not consumed in coagulation and substitution of the patient's serum for normal serum in the incubation mixture reveals the defect

The one stage prothrombin time is prolonged since Stuart factor as well as Factor VII and Factor V is essential for optimal development of thromboplastin activity from the usual tissue sources and platelets

Stuart factor has been shown to be vitamin K dependent and is reduce after several days of administration of coumarin drugs[34] The half life Stuart factor in the body has been reported to be 2.85 days[4,5]

Clinical Findings The patients described have had a moderate bleedi diathesis Though hemorrhagic symptoms may occur in childhood clinic manifestation of the disorder may not appear until later in life Freque epistaxis and hematoma have been most disturbing Hemarthrosis has bee reported but is mild and infrequent

While the defect is most severe in the homozygote it is important to no that heterozygotes with hemorrhagic symptoms have been reported. The patients usually female have been noted to be bad operative risks and have persistent anemia presumably secondary to menorrhagia

The bleeding time tourniquet test and platelets are normal Fibrinoge is quantitatively and qualitatively normal The whole blood clotting tim and thromboplastin generation test are abnormal due to deficient formatio of blood thromboplastin activity One stage prothrombin time is prolonge as Stuart factor is required for evolution of optimal tissue thromboplast activity

Treatment Transfusion with whole blood or plasma is effective relieving symptoms As Stuart factor is relatively stable it is not necessa to use freshly prepared material The general management of these patien is similar to that of those with antihemophilic factor deficiency

DISORDERS AFFECTING PREDOMINANTLY THE SECOND STAGE OF BLOOD COAGULATION

The following disorders affect predominantly the second phase of bloo coagulation and result in an abnormal one stage prothrombin time tes While congenital prothrombin deficiency is recognized, it is extremely rar Many cases formerly thought to represent congenital deficiency of prothron bin have on retesting in light of present knowledge proved to be deficienci of Factor V Stuart factor or Factor VII

Factor VII Deficiency

In 1951 Alexander Goldstein Landwehr and Cook reported the fir patient recognized to have a congenital deficiency of Factor VII[9,4] Th characteristic defect noted was a prolonged one stage prothrombin tim corrected by normal plasma or serum but not by $BaSO_4$ treated plasma Sin the two stage prothrombin time was consistently normal it was recognize that the defect could not be due to a prothrombin deficiency As BaSO treated plasma contains Factor V its failure to correct the prolonged on stage prothrombin time established the syndrome as distinct from Owren Factor V deficiency as well

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Prothrombin Deficiency

In 1934 Dam and Schonheyder[13, 3] noted that chicks raised on an arti ficial diet had a hemorrhagic diathesis unaffected by ascorbic acid The following year Dam[31] proposed the name vitamin K for the antihemorrhagic factor deficient in the diet and noted that it was fat soluble Many com pounds have been found to have vitamin K activity All of these compounds are essentially derivatives of 2 methyl 1 4 napthaquinone Vitamin K_1 was isolated from alfalfa and vitamin K from putrefying fish meal.[1, 4] They differ from the synthetic menadione (2-methyl 1 4 napthaquinone) by having a group substituted in the 3-position and from each other by the nature of the group Vitamin K_1 which is 2-methyl 3-phytyl 1 4 naptha quinone and menadione are both used clinically

During this same period a number of workers were investigating the hemorrhagic sweet clover disease in cattle Although first thought to be of infectious origin it was soon recognized to result from the ingestion of spoiled sweet clover The agent in spoiled sweet clover responsible for the hemorrhagic tendency was identified[6, 1] as 3 3 methylene bis (4 hy droxycoumarin) and its effect on the prothrombin time test demonstrated [106] This entire story is reviewed in excellent fashion by Link. [7]

A number of "coumarin drugs" have now been synthesized and are used in the prevention of thrombosis The structural relationship between the coumarin drugs and synthetic vitamin K is striking and it has been sug gested that these anticoagulants may compete with vitamin K and substitute for it in reactions ordinarily leading to the formation of prothrombin They act then in the manner of antimetabolites preventing normal utilization of vitamin K [64] More recently it has been noted that Factor VII the Stuart factor [6] and probably PTC as well require vitamin K for their synthesis and are quantitatively reduced by the action of the anticoagulant coumarin derivatives [64]

Pathologic Physiology While congenital hypoprothrombinemia proba bly exists it must be extremely rare Instances of this disorder reported before 1957 must be reevaluated in light of recent advances in our understanding of the evaluation of thrombin The present discussion will be confined to the acquired form of the disorder

It is not possible to produce a significant dietary deficiency of vitamin K in man as the intestinal bacteria produce sufficient vitamin to cover the daily needs A deficiency will become apparent in any situation wherein the vita min cannot be absorbed from the intestinal tract, however Vitamin K is fat soluble and its absorption from the intestinal tract is impaired by disorders which result in a decrease in bile acids and consequent decrease in fat ab sorption [107] Loss of large quantities of fat in the stool will likewise lead to loss of the vitamin The occurrence of vitamin K deficiency in patients re ceiving wide spectrum antibiotics over long periods of time with sterilization of the intestinal tract and loss of vitamin K producing bacteria has been reported when associated with a vitamin K-deficient diet.

Since 1951 a number of case reports have appeared in the literature described as Factor VII deficiency All had prolonged one stage prothrombin times uncorrected by $BaSO_4$ treated plasma but results of other coagulation tests varied Recent work[60] has now established a third factor (Stuart factor) necessary for optimum development of thromboplastin activity under the conditions of the one stage prothrombin time test Some of the cases formerly designated Factor VII deficiency have now been reexamined and found to be deficient not in Factor VII but in the newly described Stuart factor Much of the confusion in the literature prior to 1957 relative to Factor VII deficiency can now be resolved

Pathologic Physiology The mode of inheritance of congenital Factor VII deficiency is not known for certain but the gene is thought to be an autosomal recessive[49] It seems clear from the studies carried out on the blood of patients with Factor VII deficiency that this factor while necessary for optimal development of extrinsic thromboplastin activity is not required for the development of intrinsic thromboplastin activity Normal clotting time and thromboplastin generation test are therefore understandable The presence of hemorrhagic symptoms in these patients attests to the importance of the development of optimal extrinsic thromboplastin activity in the physiologic defense of hemostasis[4]

Factor VII is thought to be formed in the liver and is vitamin K dependent It may be diminished in severe liver disease and is rapidly reduced during the administration of coumarin drugs[34] Recent studies[4] indicate the half life of Factor VII in normal man to be 7 2 to 10 8 hours The clinical and laboratory findings in acquired Factor VII deficiency would of course reflect the primary disorder in addition to presenting those of the congenital deficiency

Clinical Findings Patients with Factor VII deficiency may have the onset of abnormal bleeding in infancy and hemorrhage from the cord has been noted Gastrointestinal hemorrhage easy bruising and frequent epistaxis are common These patients may also have mild hemarthroses The tourniquet test and platelets are normal In the majority of the patients bleeding time is normal but in as many as one third it may be prolonged This finding cannot be explained at present The thromboplastin generation test is normal in Factor VII deficiency but abnormal in Stuart factor deficiency The one stage prothrombin time is prolonged and not corrected by the addition of $BaSO_4$ treated normal plasma (containing Factor V) The laboratory findings of acquired Factor VII deficiency will of course reflect the underlying pathology in addition

Treatment Administration of fresh blood or plasma to patients with a congenital defect of Factor VII has been effective The effects are short lived and the prothrombin time returns to pre transfusion levels within 24 hours[4]

Factor VII deficiency secondary to anticoagulant coumarin drugs will respond to vitamin K_1 administration

cutaneously intramuscularly or intravenously A 2 ml ampule containing 75 mg. is available for intravenous administration in emergency situations

Illustrative Case

This 10 year old child was admitted to the hospital for investigation of a hemorrhagic diathesis At birth she had a severe hemorrhage from the umbilicus and over the succeeding years recurrent episodes of epistaxis gastrointestinal hemorrhages and multiple hematomas Excessive bleeding often followed minor trauma but hemarthroses had not occurred

Physical examination revealed only the presence of multiple bruises on the lower legs

LABORATORY EXAMINATION Erythrocyte count 4 58 mil per cu mm leukocyte count 9880 per cu mm hemoglobin 10 5 gm per 100 ml Tourniquet test bleeding time clot retraction and coagulation time were normal Platelet count (indirect) 261 000 per cu mm Quick one stage prothrombin time was 54 seconds with a control of 14 seconds

On her first admission at the age of 5 years it was thought that she had idiopathic hypoprothrombinemia on the basis of the data then available

During a subsequent admission it was found that when normal plasma was mixed with that of the patient the prothrombin time was reduced but did not return to normal The addition of "purified prothrombin did not result in a normal one stage prothrombin time One part of serum when mixed with three parts of the patient s plasma resulted in a normal one stage prothrombin time On the basis of these studies it was felt that the patient probably had a Factor V deficiency

The patient was then followed at another hospital where it was realized that she could not represent a Factor V deficiency since the one stage prothrombin time was corrected by the addition of normal serum and not by normal plasma Factor VII was described at that time and it was noted that the clinical and laboratory findings of this patient fitted well with those of the Factor VII deficiency state She was then carried as a Factor VII deficiency until the thromboplastin generation test became available Once again the patient was reevaluated It has now been concluded that a deficiency of Stuart factor is responsible for her hemorrhagic diathesis

COMMENT This case illustrates the growth of our knowledge of the second stage of blood coagulation The patient was known to have abnormal bleeding which was not related to platelet or vessel factors She was studied extensively with all of the tests available during each hospitalization A diagnosis of idiopathic hypoprothrombinemia was made when she was first admitted on the basis of the Quick one stage prothrombin time test It was not until the thromboplastin generation test became available and it was found that the substitution of this patient s serum for normal serum in the incubation mixture resulted in a reduction in thromboplastin activity that the patient could be identified as having a Stuart factor defect

Severe liver disease may also result in an abnormal prothrombin time In these cases there is an adequate amount of the vitamin but the reactions in which it takes part in the liver are impaired

The administration of anticoagulant drugs may produce the same effects as vitamin K deficiency In addition to the coumarin drugs salicylates are known to produce hypoprothrombinemia. The mechanism by which these compounds exert their effect is not clear [8] but it has been suggested that it is similar to that of the coumarin drugs

Prothrombin Factor VII PTC and Stuart factor levels are below normal during the first six weeks of life [1] - While most investigators have found a rise in these factors following the administration of vitamin K to the mother in labor or to the newborn infant others[38] have failed to achieve this result It has been suggested[143] that the low concentration of these factors in the neonatal period could be responsible for some instances of hemorrhagic disease of the newborn [1] Though this point is not yet settled[38] it is common practice to administer a routine prophylactic dose of 1 or 2 mg vitamin K to all infants at birth Large doses of vitamin K (over 5 mg) have been reported to increase serum bilirubin levels and the risk of kernicterus in premature infants and should be avoided [87]

Clinical Examination Hypoprothrombinemia may result in a hemorrhagic diathesis manifested by bleeding from the gums nose or gastrointestinal tract Excessive menstrual flow is also a common finding

A prolongation of the one stage prothrombin time is found in these disorders The thromboplastin generation test should be normal and the modified thromboplastin generation test will indicate a deficiency of prothrombin Fibrinogen assays are normal

Treatment Blood transfusions should be given in emergency situations and will serve to treat shock correct anemia and aid in restoration of missing coagulation factors

It has now been found [118] contrary to the original report [11] that natural vitamin K_1 (2-methyl 3 phytyl 1 4 naphthoquinone) is more effective than the synthetic vitamin K (menadione)

Vitamin K_1 is given slowly by the intravenous route in severe deficiencies when bleeding is imminent or has occurred The time needed for a response to the drug to be demonstrable depends on the cause of the deficiency and the amount of vitamin administered Generally bleeding is controlled in 3 to 6 hours A 1 ml ampule contains 50 mg vitamin K_1 It is recommended that it be administered slowly at a rate not exceeding 10 mg/min

Vitamin K_1 is also available in 5 mg tablets for oral administration in nonemergency situations Five to 10 mg are given initially and the dosage adjusted depending on the clinical situation In the presence of obstructive jaundice or biliary fistula the concomitant administration of bile salts is necessary when the drug is given by mouth

Synthetic vitamin K (menadione) supplied as the sodium salt of 2 methyl 1 4 naphthohydroquinone is a water soluble compound It is supplied in 1 ml ampules containing 5 or 10 mg It may be administered sub

on the plasma fibrinogen level than will subsequent doses of comparable size Transfusions of whole blood should be given to replace blood loss if need be[104]

Acquired Fibrinogen Deficiency

Pathologic Physiology The mechanism by which the blood becomes depleted of fibrinogen in these conditions has been extensively investigated Decidual and placental extracts are both rich tissue sources of thromboplastin activity Widespread intravascular clotting may follow if these tissues or those of the lung or brain should gain access to the vascular system This clotting is so widely disseminated that obstruction to the flow of the blood may not occur but the utilization of fibrinogen by the coagulation process will dangerously reduce its level[116] In a like manner the level of fibrinogen has been thought to be reduced by the coagulation of large retroplacental hemorrhage in instances of abruptio placentae[10] Fibrinolysins have been reported to occur in some obstetrical conditions[16, 110, 111, 147] and in prostatic carcinoma[14] and may be responsible for the depressed levels of fibrinogen

Sharp et al[1 9] have recently noted that a portion of the plasma fibrinogen may be physiologically inert during the early phases of this syndrome Laboratory methods which quantitatively measure the level of fibrinogen may not be adequate and a test which measures the reactivity of the fibrinogen to thrombin is preferred

Clinical Examination Acquired fibrinogen deficiency may develop suddenly during labor or shortly thereafter and is manifested by generalized bleeding and incoagulability of the blood It has been seen following abruptio placentae[116, 147] in association with amniotic fluid emboli[111] and with the retention of a dead fetus[110] It is important to emphasize the suddenness with which this syndrome may arise Its early recognition is imperative The condition has also been reported following pulmonary resections in association with carcinoma of the prostate following incompatible blood transfusions and with severe burns[1, 13, 136]

Laboratory tests commonly used to demonstrate fibrinogen deficiency employ the fibrin clot as an end point In the presence of fibrinogen deficiency the clot fails to form or is of poor character The thrombin titer is perhaps the most reliable test Normal plasma when diluted 1 to 64 with saline and often in the 1 in 128 dilution will clot following the addition of thrombin Plasma from patients with fibrinogen deficiency often fails to clot even at low dilutions The whole blood clotting time is often abnormal When the blood clots in the normal period of time the clot is usually of poor quality but in some instances no coagulation occurs

The one stage prothrombin time though a sensitive indicator of fibrinogen deficiency is non specific Deficiency of Stuart factor Factor V Factor VII or prothrombin will also give abnormal results in this test The thrombin clotting time of plasma is often prolonged (normal 10 to 12 seconds) The

DISORDERS AFFECTING THE THIRD STAGE OF BLOOD COAGULATION

Fibrinogen deficiency may be either congenital or acquired The congenital variety is quite rare Acquired fibrinogen deficiencies are more common and usually present as complications of certain surgical procedures or obstetrical accidents and are of grave importance

Congenital Fibrinogen Deficiency

Pathologic Physiology Congenital fibrinogen deficiency appears to be genetically transmitted as a non sex linked recessive In many of the reported cases the family history revealed consanguineous marriages in parents or grandparents [43, 8, 104]

In these cases the absence of fibrinogen prevents the coagulation process from completing its final phase i e the conversion of fibrinogen to the fibrin clot Thus coagulation cannot occur The arrest of small vessel hemorrhage is normal as it is brought about by vasoconstriction and platelet thrombi [3] Menstrual bleeding is normal and seems to be controlled by vasoconstriction of endometrial arterioles These patients have been shown to have a decrease in synthesis of fibrinogen rather than an increase in its destruction Minute traces of fibrinogen have been detected immunochemically in the blood and connective tissues of some of these patients and the defect may not be absolute in all instances [43] Others have been unable to detect fibrinogen by any method [3]

Clinical Examination The disorder is often manifested at birth by the occurrence of bleeding from the umbilical cord [3, 8, 104] The condition resembles AHF deficiency clinically but is usually less severe [10] Persistent bleeding may occur however in the wake of minor trauma and spontaneous bleeding may occur into joint spaces or soft tissues Crippling hemarthroses do not occur in this disorder [8, 104] The thromboplastin generation test is normal since there is no defect in the early phases of clotting Platelet count tourniquet test and bleeding time are likewise unaffected by the absence of fibrinogen The whole blood clotting time one stage prothrombin time and thrombin time all require a fibrin clot as an end point and are abnormal in the absence of fibrinogen Fibrinogen assay reveals only minute traces of fibrinogen or a complete absence

Treatment The hemostatic defect in patients with congenital fibrinogen deficiency can be corrected by the administration of fibrinogen Half of the fibrinogen administered intravenously to patients with congenital afibrinogenemia has been reported to be lost in 48 hours and thereafter it disappears with a half life of approximately four days [6] The early loss of half of the administered fibrinogen is thought to represent equilibration with the extravascular compartment [43] The mass of plasma protein located in extravascular spaces is approximately equal to the mass of plasma protein circulating within the blood vessels and the two are in dynamic equilibrium As the extravascular deficit must be made up as well as that of the intravascular compartment the first dose of fibrinogen administered will have less effect

discovered In this case the house officer noted that the patient's blood did not clot and when the addition of thrombin to the patient's plasma did not bring about coagulation the administration of fibrinogen was immediately begun The patient's response to fibrinogen was good It is interesting to note that the administration of 3000 ml of whole blood which contain approximately 9 gm of fibrinogen did not correct the defect while the administration of 4 gm of fibrinogen in saline brought about a distinct decrease in the rate of hemorrhage The reason for this discrepancy which has been noted previously is unknown The bleeding which occurred at the time of delivery of the stillborn infant was probably related to the tear rather than to fibrinogen deficiency since the coagulation time was still normal

In retrospect it would have been advisable to avoid the administration of dextran to this patient since dextran itself may interfere with the clotting mechanism [119]

III DISORDERS OF BOTH VESSEL FUNCTION AND BLOOD COAGULATION

Von Willebrand's Disease (Inherited autosomal hemorrhagic diathesis with antihemophilic factor deficiency and prolonged bleeding time)

In 1926 von Willebrand described a hemorrhagic diathesis found in members of a family living on the Aland Islands off the coast of Finland He designated the condition as pseudohemophilia and felt that it resulted from abnormal platelet function A number of reports of cases of pseudohemophilia have since appeared in the literature The patients reported generally have had clinical findings similar to those of the family described by von Willebrand but the results of the laboratory tests have been varied It now seems apparent that the term pseudohemophilia as used in the literature is not specific Reassessment of the patients originally described by von Willebrand has made clear the nature of their defect[9] and it seems reasonable to refer to their disorder as von Willebrand's disease

Pathologic Physiology Von Willebrand's disease is inherited and is transmitted as an autosomal dominant with varying degrees of expressivity [9, 94] The patients originally described by von Willebrand and thought to have a qualitative platelet defect have been reexamined [9] and no such defect could be established It would seem reasonable to include under the term von Willebrand's disease only those patients who have findings similar to those of the original group namely a prolonged bleeding time slightly prolonged coagulation time moderate or slight decrease in AHF levels and normal platelets It has been demonstrated that the AHF level may be raised by the administration of fraction 1-0 of normal human plasma and the bleeding time simultaneously is corrected The bleeding time can also be corrected by the administration of a fraction 1-0 which has no AHF activity

thrombin time is less sensitive than the thrombin titer however Acquired fibrinogen deficiency may be associated with a deficiency of Factor V AHF and platelets The platelet count is often low during the acute phase of the syndrome [11] AHF and Factor V deficiency can be demonstrated by the thromboplastin generation test

Treatment Immediate treatment is often required in these cases Fibrinogen preparations are administered in doses of 4 to 6 gm and should be repeated as needed Steroids may be of benefit if fibrinolytic activity is present [3] Steps should be taken to correct the underlying disease if possible Fibrinogen alone may not be adequate as there may be associated defects of Factor V and AHF and fresh whole blood should be used as well

Illustrative Case HISTORY A 40 year old white woman gravida 10 para 8 aborta 1 was admitted one month prior to the expected date of delivery She had progressed normally in her gestation until the night before admission when she developed diarrhea and weakness The patient fainted on the morning of admission and her physician was called He found her in shock and referred her for treatment

PHYSICAL EXAMINATION Examination revealed a blood pressure of 30/0 with a pulse rate of 120 per minute A term fetus was present with the vertex floating The uterus was very firm and the fetal heart could not be heard There was no external loss of blood

LABORATORY EXAMINATION The hematocrit was 22 per cent and urinalysis revealed 6 to 8 w b c /hpf 20 to 30 r b c /hpf and 4+ albumin A diagnosis of premature separation of the placenta with non viable fetus was made

COURSE As the patient was moved from the stretcher into bed approximately 500 ml of unclotted blood gushed from the vagina An intravenous infusion of 500 ml of dextran was started immediately and within a few minutes the administration of 500 ml of whole blood was started Six bottles of whole blood (3000 ml) were necessary over the next one and one half hours to maintain the blood pressure above shock levels because of continued and profuse vaginal hemorrhage It was noted that the patient s blood did not clot and a fibrinogen assay was performed The addition of human thrombin to her plasma did not result in coagulation which indicated the probability of a fibrinogen deficiency Four grams of fibrinogen in 400 ml of saline was administered intravenously Three additional pints of blood (1500 ml) were infused in the next hour Since bleeding continued though somewhat slowed an additional 2 gm of fibrinogen was given intravenously in 200 ml of saline following which the coagulation time was four minutes Patient was delivered of a stillborn infant 18 hours after admission with the loss of approximately 700 to 800 ml more blood and additional whole blood was administered Coagulation time at this point was still normal Following repair of a small laceration of the fourchette bleeding ceased and the remainder of the patient s course was uneventful

COMMENTS Excessive bleeding is expected with premature separation and often it is some time before the existence of a fibrinogen deficiency is

age when Gelfoam and topical thrombin became available and an extraction was successfully carried out using these agents She was now admitted for further dental work, having had but one nosebleed of any significance in seven years and only rare vaginal spotting

The patient's mother and father were first cousins There was no history that either the parents or grandparents had had any abnormal bleeding Two brothers had died following hemorrhage in infancy another brother died following uncontrollable epistaxis and a fourth brother bled to death during an appendectomy A fifth committed suicide The remaining five siblings were alive and well without historical evidence of abnormal bleeding

A review of the old record revealed marked variability of the bleeding time and the fact that the coagulation time was somewhat prolonged occasionally Prothrombin time platelet counts and clot retraction were consistently normal The results of the tourniquet test varied and from time to time were slightly positive *On one occasion the bleeding time was noted to* be 17½ minutes in one ear while at the same time it was 180 minutes in the other There was also striking evidence that the infusion of whole blood *affected the bleeding time On one occasion the capillaries were visualized* directly and it was noted that they did not retract properly after being punctured with a needle

PHYSICAL EXAMINATION In addition to dental caries for which she was admitted the only positive physical finding was very slight hepatomegaly

LABORATORY FINDINGS The red blood cell count was 3 5 mil per cu mm hemoglobin 9 6 gm per 100 ml hematocrit 29 per cent and platelets 364 000 per cu mm The bleeding time was discontinued at 15 minutes the coagulation time was 13 minutes and the thromboplastin generation test revealed a depression of AHF

COURSE The patient was given prednisone and 3 units of fresh (4 to 6 hours old) blood Her bleeding time and thromboplastin generation test returned to normal and she tolerated the removal of three teeth without excessive bleeding Within 48 hours the bleeding time was again prolonged despite continued administration of prednisone Five hundred ml of fresh plasma in glass bottles was given and once again the bleeding time was returned to normal She tolerated the removal of two more teeth without excessive bleeding

COMMENT The history presented indicates the variability of the clinical manifestation of this disorder There is clear evidence that the bleeding time was affected by both whole blood transfusions and plasma transfusions The fact that plasma transfusions collected in and administered from glass bottles *corrected the defect affords evidence against the possibility that platelets* played any role in the correction of the bleeding time The patient's platelets were normal in the thromboplastic generation test The decreased AHF and prolonged bleeding time indicates the *similarity of this patient's disorder to* those described by von Willebrand though this patient's defect may have been inherited as an autosomal recessive The clinical course of this patient was favorably altered by the administration of fresh plasma The administra

Fibrinogen has no effect on the bleeding time These findings suggest that the fraction I–0 of normal plasma contains a factor other than AHF or fibrinogen which will influence the bleeding time The identification of this factor has not yet been accomplished [9, 93]

Clinical Features This disorder is often apparent in the first few weeks of life when spontaneous bleeding from the nose or gums may occur Excessive bleeding often follows minor trauma Uterine bleeding may be excessive and pregnancy is often complicated by bleeding episodes [93, 1, 1] Neither petechiae nor hemarthrosis is common in this disease The great variability in the bleeding tendency in the same patient from time to time makes the clinical course difficult to anticipate but it is rare for these patients to succumb during an episode of bleeding

The laboratory findings in this group of patients are not consistent from patient to patient or indeed in the same patient from one examination to the next [8, 0, 93, 118, 1, 1]

The platelets are normal quantitatively and qualitatively The one stage prothrombin time is normal since it bypasses the defect Fibrinogen levels are adequate The bleeding time is prolonged The coagulation time may be prolonged and seems to reflect the level of AHF as does the thromboplastin generation test which is abnormal in the plasma phase In 1941 Macfarlane[83] demonstrated that the capillaries in the nail beds of these patients appeared bizarre and failed to constrict normally

Treatment The fact that more than one disorder has in the past been included under this name may be responsible for the wide variation reported in response to therapy

Blood transfusions are indicated for support of a patient through a severe hemorrhagic episode The use of fresh plasma has been effective in control as has Cohn s fraction I–0

The natural history of this disorder is extremely variable and it is difficult to interpret the efficacy of a particular therapeutic maneuver Any surgery is a major hazard to these patients and should be avoided if possible If surgery is unavoidable the patient should be prepared by the administration of fresh plasma

Illustrative Case HISTORY A 50 year old white married woman was admitted to the hospital for the extraction of several teeth She had been known to be a "bleeder all her life Minor cuts and abrasions were followed by excessive bleeding in childhood and there had been repeated epistaxis subcutaneous hemorrhages and oozing from the gums At 18 years of age she had a severe hemorrhage which continued for two to three days following the extraction of a tooth Following a miscarriage at the age of 33 she had intermittent bleeding for six months Bleeding was finally controlled by the administration of a number of whole blood transfusions There were however long intervals of freedom from any evidence of bleeding tendency

She began to have severe pain as the result of excessive dental caries while in her late thirties Dental extractions were denied her on several occasions because of her history of a hemorrhagic diathesis She was 42 years of

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tion of adrenocortical steroids was not considered to have played a role in the correction of the bleeding time since it was prolonged again 48 hours after administration of fresh whole blood despite their continued administration In retrospect the use of dental splints as described under the treatment of AHF deficiency may have obviated some of this patient's difficulty with extraction

Thrombopathia

Hemorrhagic diathesis has been attributed to qualitative as well as quantitative changes in the platelets [6, 14, 139, 140] As the platelets play so broad a role in the defense of hemostasis altered platelet function may affect the vascular or biochemical phase of hemostasis or both It is not surprising to find a wide range of abnormal laboratory tests reported in the literature since individual platelet functions are difficult to assess and more than one function may be altered

Thrombopathia is usually investigated by the use of the thromboplastin generation test heparin neutralizing activity clot retraction morphologic study of the platelets bleeding time and tourniquet test

At present it seems clear that altered platelet function may produce hemorrhagic diathesis but clarification of these platelet abnormalities must await further investigation

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Leukocytes

CREDIT FOR distinguishing between the red and white corpuscles in the circulating blood has been given to William Hewson [11] The term "leukocytosis" was introduced by Virchow to indicate a temporary increase in the number of circulating leukocytes he recognized that increases occurred in a variety of normal and disease states The association of leukocytosis with infectious processes attracted considerable attention and stimulated numerous investigations Almost fifty years ago the authors of one book on hematology wrote "The problem of leukocytosis has been subjected to as much discussion as any question of modern medicine An exhaustive recital of the work devoted to it of its methods and of the results of this work would fill a whole volume and would be out of place in a treatise on blood diseases" [6] Since that time other investigations of the origin function and variations in the leukocytes have added to our knowledge but many important details remain to be clarified

The origin morphology and function of the leukocytes are discussed in Chapter I *The present chapter deals with the numbers of the different types* of leukocytes that are found in normal circumstances the variations that occur under different physiologic and pathologic conditions and the mechanisms that are considered important in producing these changes

LEUKOPOIESIS

Granulocytes

The granulocytes constitute a tissue of considerable size. Only a small part of this tissue is contained in the circulating blood where the number of granulocytes normally ranges from 2000 to 6000 per cu mm. Osgood has calculated that the total number of the cells of this series in the hematopoietic organs (marrow) is 18×10^{11} and in the circulating blood 20×10^{9} in the tissue exclusive of the blood and blood forming organs the calculated total is 12×10^{11}.[4] The quantity and distribution of the cells are better appreciated by considering the total mass of cells rather than the total numbers. *It is estimated that the weight of the granulocytic cells in the marrow* is 900 gm in the circulating blood 10 gm and in other tissues 600 gm. In the normal adult the spleen weighs about 150 gm and the liver about 1500 gm.[1] The presence in the circulation of only a relatively small percentage of the total number of granulocytes in the body supports the interpretation that the cells in the blood stream are those that are being transported from one site to another.[4]

The location and function of the extravascular granulocytes is an intriguing problem. The bone marrow apparently serves as a reservoir of functioning cells as well as an organ of production.[4, 37] Other sites of extravascular accumulation are the lungs liver and spleen.[1, 4] Granulocytes may also be found within the vascular compartment arranged along the walls of the capillaries in various tissues apparently sequestered from the general circulation. *The presence of cells in these locations may indicate* that they perform some function such as transportation or it may indicate that the cells accumulate in these areas prior to elimination or destruction. It is also possible that these intravascular aggregates of cells constitute a reserve of motile cells ready to be utilized in the defense of the body. Experimental evidence indicates that leukocytes that have left the intravascular compartment are unable to reenter in significant numbers.

The studies of Craddock and coworkers have provided valuable information about the location and size of the reserve pool of granulocytes.[3, 4, 7] *Dogs were subjected to the rapid removal of leukocytes by leukopheresis* and the responses were studied under various conditions. The tremendous reserve of leukocytes was demonstrated by the observation that it was necessary to remove the leukocytes from the blood at a rate of 1.5 blood volumes per hour for three hours in order to reduce the leukocytes to a level of 1000 to 1500 per cu mm. When the initial leukopheresis was terminated the leukopenia persisted for about two hours (phase I). Following this delay a linear rise in the leukocyte count occurred and within a period of six to eight hours the count reached a level that was 150 to 200 per cent of the pre leukopheresis baseline (*phase II*). *This phase was followed by a stabilization* period at the new level that persisted for from 24 to 36 hours (phase III). If leukopheresis was carried out during phase II the period of greatly

accelerated entry of leukocytes into the peripheral blood the procedure could be carried out for long periods without the production of leukopenia Leukopheresis during phase III and for two or three days thereafter produced a response similar to that observed initially Morphologic studies of the leukocytes in the peripheral blood and bone marrow revealed no evidence of increased immaturity of the granulocytes until after the third or fourth day The data indicate that the response to leukopheresis during this period came from preformed cells that were available in areas of storage Further support for this interpretation was afforded by the observation that dogs subjected to LD $_{50}$ whole body irradiation behaved similarly under the same conditions although the magnitude of the response was less

In another series of experiments the responses of labeled leukocytes were studied by the determination of leukocyte DNA in which radioactive phosphorus had been incorporated The cells labeled in this manner did not appear in the peripheral blood in appreciable numbers until three or four days after the leukopheresis experiments were performed Leukopheresis performed on the third day after the marrow cells had been labeled with DNA P^{32} indicated that a high percentage of the cells which contributed to the rapid rise during phase II came from the marrow leukocyte pool

From these studies it was concluded that the main source of mature granulocytes that take part in an acute leukocytosis is the marrow reserve The mechanism whereby the stimulus of leukopheresis is mediated to the marrow is unknown The results obtained with leukopheresis were not interpreted to indicate that in all circumstances leukocytosis occurs only as a result of the release of cells from the marrow and not from redistribution of leukocytes within the vascular system These studies provide new information about the mechanism of leukocytosis and emphasize the importance of the large reserve pool of granulocytes in the marrow described by Kindred [13] Yoffey [37] and Osgood [4]

The factors that control the level of the granulocytes in the circulation in normal and abnormal conditions have not been defined clearly Infection has been recognized as a stimulus to neutrophilic leukocytosis from the time that leukocytosis was first described In Ehrlich s and Lazarus textbook of fifty years ago chemotaxis was accepted as the explanation of the migration of leukocytes into areas of infection and it was postulated that the marrow cells were stimulated by a chemical that led to increased proliferation and output of these cells [6] The importance of the reactive ability of the marrow was recognized for it was known that an injection of oil of turpentine produced leukocytosis in normal individuals but failed to do so in patients with typhoid fever and leukopenia until after the fever and leukopenia had disappeared More recent studies have provided a better understanding of some of the factors concerned in the leukocytosis of infection Nettleship concluded that the substance responsible for hyperplasia in the marrow was released from necrosing leukocytes [2] Menkin and associates have demonstrated the presence of a "leukocytosis promoting" factor in inflammatory exudates in animals and man This factor which is thermolabile was also found in the

circulating blood of dogs when inflammatory exudates were produced in the pleural space by the instillation of turpentine but it could not be demonstrated in normal blood or serum[16, 1, 18, 19] The leukocytosis induced by the injection of this substance was accompanied by proliferation of the younger forms and increased cellularity in the marrow Efforts to produce similar alterations in the leukocytes by the intravenous injection of histamine nucleic acid derivatives adenosine or bacterial suspensions were unsuccessful The leukocytosis promoting factor appears to be distinct from those that are concerned with the migration of leukocytes (leukotaxin) and the necrosis of tissue (necrosin) Injection of this fraction into animals was followed by an increase of between 70 and 400 per cent in the leukocyte count within a few hours

At least in special circumstances hormonal factors exert an influence on the circulating leukocytes[8] It has been shown that ACTH produces an increase in circulating neutrophils and a decrease in circulating eosinophils[5] the similar response that has been observed in adrenalectomized animals may have been due to contamination of the ACTH material with pituitary growth factors that are known to produce a rise in neutrophils[8] The administration of cortisone to mice was followed by an increase in the myeloid cells of the marrow associated with an increase in circulating neutrophils[9]

Although most of the studies indicate that neutrophilic leukocytosis is produced by the rapid release of granulocytes into the circulation followed by the augmented production of these cells in the marrow an increased life span of the cells in the circulation occurring as a consequence of decreased removal or destruction of the cells could produce the same result The life span of the neutrophils has been estimated by different methods and different results have been obtained[14] Studies that employed transfusion or cross circulation techniques generally indicate a short life span measured in hours while other methods indicate a longer survival The marrow culture method has indicated that the total life span of the neutrophil is four and one half days[4] By the same technique the life span of eosinophils and basophils outside the marrow has been found to be from eight to twelve days while the time spent within the marrow was found to be the same as that of the neutrophils[4]

In another study when P^{32} was administered as Na_2HPO_4 to human subjects the subsequent determination of the DNA content of leukocytes at intervals of 24 hours to three weeks indicated a longer life span for the granulocytes[1] It was found that the marrow showed high DNA P^{32} specific activity for three or four days before the labeled cells were found in the circulation The observations led to the conclusion that the total life span is about thirteen days four of which are spent in the marrow Another study that utilized DNA labeled with P^{32} indicated that the granulocytes generally enter the circulation at six days of age the mean age of circulating granulocytes was found to be about nine days and only a negligible number survived for more than three weeks The granulocytes in two patients with chronic myelocytic leukemia were found[10] to have an average life span of 23

days when studied by a method that employed the adenosine-8 labeled with C^{14} The longer life spans found with the isotope labeling technique are probably nearer to the true value than the shorter ones obtained in transfusion and cross circulation experiments

If the neutrophil is not destroyed in the circulation it eventually leaves the blood stream and migrates into the extravascular spaces Some authors believe there is a free two way flow of leukocytes between the circulation and the extravascular space[1] while others do not think there is any significant reentry into the circulation from the extravascular tissue[4, 7] The sites most concerned in the removal of leukocytes from the circulation are the lungs liver spleen gastrointestinal tract, bone marrow striated muscle and kidney[1] Studies that employed catheterization of various vessels have revealed that the lungs remove large numbers of leukocytes from the circulation The removal of cells from the peripheral circulation could be initiated by the administration of histamine nicotinic acid, and colloidal substances The fate of the cells trapped in the pulmonary circulation is uncertain but the following possibilities have been suggested (1) transient trapping with later entry into the general circulation (2) migration and excretion through the sputum and gastrointestinal tract (3) reentry into the general circulation by way of the lymphatics and thoracic duct (4) disintegration in the lung[1] A failure of the removal mechanism has been suggested as a cause of leukocytosis in certain diseases especially leukemia

Lymphocytes

Only a small percentage of the lymphocytes in the body are present in the circulating blood. Osgood has calculated that the total numbers of lymphocytes are as follows bone marrow 4×10^{11} (100 gm) lymphocytic tissue and spleen 4×10^{11} (100 gm) peripheral blood, 12×10^{9} (3 gm) total outside the blood and blood forming organs 52×10^{11} (1300 gm)[4]

Although the injection of ACTH has been shown to cause a lymphocytopenia followed by a lymphocytosis[5] the factors that control the level of these cells in the circulation under normal circumstances are poorly understood The life span of the lymphocytes in normal individuals and those with chronic lymphocytic leukemia has been calculated to be at least 30 days one tenth of which is thought to be spent in lymphatic tissue[4] Studies that employed the labeling of lymphocytes with radioactive isotopes in man suggest a dual population of lymphocytes one with a mean age of two to three days and a larger number (80 per cent) with a mean age of 100 to 200 days[5] Similar investigation of two human subjects with chronic lymphocytic leukemia indicated that the lymphocytes had an average life span of 85 days There was also prolonged retention of isotope in other cells (half time about 300 days) but whether this indicated a dual population of cells one of which survived for very long periods or a specific reutilization of large fragments of nucleic acid or nucleoprotein was uncertain[10]

The function and fate of the lymphocytes that enter the blood stream

each day is one of the interesting problems in hematology The lymphocytes are produced in the lymphoid tissue and enter the blood through blood capillaries or lymphatics[3] Although the life span of the lymphocyte has been estimated as more than 30 days [4] studies in animals that utilized a cannula in the thoracic duct indicate that lymphocytes enter the blood stream at a rate sufficient to replace the total in the circulating blood from two to twenty four times each day[9 37] Yoffey considers that the evidence is against the existence of a true circulation of the lymphocytes between the blood and lymph and for this reason interprets the discrepancy between the levels of cells in the circulating blood and the outflow from the thoracic duct as evidence that large numbers of small lymphocytes leave the circulating blood probably to enter the bone marrow the lumen of the intestine and the connective tissue throughout the body[37] However Gowans who transfused labeled lymphocytes into the veins of rats and studied the composition of the cells obtained from the thoracic duct of the recipient rats concluded that the great majority of cells in the thoracic duct are cells that have recirculated from the blood[9] Although the nature of the connection between the blood and the lymph remains unknown it was suggested that recirculation occurred through the lymph nodes

Monocytes

Monocytes are found normally in the lymph nodes spleen bone marrow and loose connective tissue[34] and they constitute from 3 to 7 per cent of the circulating leukocytes The total number of cells of the monocytic series in the circulating blood has been calculated to be 0 5 x 10^{11} [4] The life span of the monocyte both in the hematopoietic organs and in the circulation is thought to be comparable to that of the granulocyte [4] The origin of the monocytes is still a matter of controversy and the factors that regulate their production are not fully understood The injection of ACTH has been found to produce a monocytopenia followed by a monocytosis[34] Monocytes occur in large numbers about early tubercles and in the early stages of inflammation The monocyte has been considered to be a young cell destined to become a macrophage and thus a cell that may develop into an epithelioid cell or a giant cell if appropriate conditions are present[34]

NORMAL VALUES

The normal values for the total and differential leukocyte counts in adults according to various authors are shown in Table 10 Both the total count and the percentages of the different cells vary with the age of the individual In the newborn total counts varying from 3600 to 45 000 per cu mm have been reported but the great majority are in the range of 10 000 to 25 000 per cu mm [7 33] at this time the neutrophils constitute about 60 per cent of the cells From the second week through the second year when the total count ranges from 8000 to 13 000 per cu mm the lymphocytes predominate

and make up from 50 to 69 per cent of the total[7, 33] During childhood the total count is usually around 8000 per cu mm and the range is 4000 to 13 000 per cu mm during the same period the percentage of lymphocytes decreases to a range of 25 to 48 per cent by the twelfth year[33] At about the fifteenth year the adult values are reached and these are maintained throughout the remainder of the individual's life

Table 10 Total and Differential Leukocyte Counts in Normal Adults According to Various Authors

AUTHOR	TOTAL (PER) (CU MM)	NEUTRO (%)	EOSIN (%)	BASO (%)	LYMPH (%)	MONO (%)
Osgood et al[43]	4 000–11 000	33–75	0–6	0–2	15–60	0–9
Sturgis and Bethell[33]	6 000– 7 400	55–67	2–3	0 5–0 7	23–38	4–8
Schilling[32]	5 000– 8 000	51–67	2–4	0–1	21–35	4–8
Wintrobe[46]	5 000–10 000	54–62	1–3	0–0 75	25–33	3–7

PHYSIOLOGIC VARIATIONS

In addition to the variations in leukocytes that are found at different ages changes occur as a result of other physiologic processes These variations which since the time of Virchow have been recognized to occur independently of disease have been the subject of several detailed reviews[33]

Spontaneous fluctuations in the leukocyte count occur during the day These appear to be inconstant irregular and unrelated to the ingestion of food[7, 8, 43] the changes have been interpreted as indicative of a redistribution of leukocytes within the body rather than an increased production of new cells because these fluctuations are not associated with an increase in immature cells[8, 7] An hourly rhythm of the leukocytes with an afternoon increase regardless of food ingestion has been reported but not confirmed[8]

Strenuous exercise produces a marked leukocytosis[7, 33] Total counts as high as 27 000 per cu mm have been found in marathon runners and football players Leukocytosis that develops within 11 seconds has also been found after a 100 yard dash After exercise the count returns to normal within several hours Although pregnancy is associated with only a slight increase in leukocytes and neutrophils[33] at the onset of labor there is a marked increase greater in primiparae than multiparae that may reach levels of 17 000 to 34 000 per cu mm[7, 33]

Other causes of leukocytosis are paroxysmal tachycardia (13 000 to 20 000 per cu mm)[7, 43] and convulsions (37 000 to 43 000 per cu mm)[7] Emotional states may be associated with changes in the leukocyte counts the count has been noted to rise from 8000 to 18 000 per cu mm with the development of a panic reaction and to decrease 6000 to 12 000 per cu mm in one hour when Amytal administered intravenously induced a change in the emotional status[9]

VARIATIONS DUE TO DISEASE

Changes that occur in the leukocytes in the peripheral blood in disease are often helpful in considering both the diagnosis and prognosis In general the changes in the peripheral blood reflect the type of injury the extent of the process and the body s reaction to the injury Significant alterations may occur in the total number of leukocytes in the proportion of the various cells present and in the morphologic appearance of the individual cells

Although no unconditional statements can be made concerning the reaction of the body to infection, certain generalizations can be stated A leukocytosis is more likely to occur in acute infections than in chronic disorders A mild infection most often is associated with no change in the leukocytes or with a slight increase to 11 000 or 15 000 per cu mm In infectious or destructive processes a marked neutrophilic leukocytosis accompanied by an increase in the younger forms indicates a severe injury and a brisk reaction of the body s defense mechanism A low or normal total leukocyte count associated with a marked increase in younger forms in the presence of infection indicates either an early stage of the infectious process or a subnormal *response to a severe infection Severe infection and extensive* tissue destruction are also manifested by morphologic changes in the granulocytes such as cytoplasmic basophilia vacuolation of the cytoplasm and toxic granulation Bacterial infections particularly those due to cocci are apt to be accompanied by an increase in the total leukocyte count and the neutrophilic granulocytes while viral infections are likely to be associated with a low or normal total leukocyte count and a relative or absolute lymphocytosis Neoplastic disease leukemia excepted is usually associated with a normal leukocyte count unless the process is extensive and tissue necrosis has occurred in this circumstance neutrophilic leukocytosis of marked degree is often present

The most common causes of increases in neutrophilic granulocytes lymphocytes monocytes and eosinophilic granulocytes are presented in Tables 11 to 14

Table 11 Causes of Neutrophilic Leukocytosis

1 Infections especially with pyogenic bacteria either localized as appendiceal abscess or generalized as septicemia ✓

2 Other disorders associated with acute inflammation or cellular necrosis infarction collagen disease acute hemolysis

3 Neoplasms leukemia carcinoma lymphomas especially with areas of tissue necrosis and wide spread metastases

4 Intoxications drugs chemicals especially in poisonings with liver damage

5 *Acute hemorrhage*

Table 12 Causes of Lymphocytosis

1	Infections	(a) Acute infectious mononucleosis pertussis infectious lymphocytosis other viral and bacillary infections (b) Convalescence especially viral infections (c) Chronic tuberculosis brucellosis syphilis
2	Neoplasms	Lymphocytic leukemia lymphosarcoma
3	Metabolic disease	Hyperthyroidism

Table 13 Causes of Monocytosis

1	Infections	(a) Bacterial tuberculosis subacute bacterial endocarditis brucellosis typhoid fever (b) Protozoal malaria, kala azar (c) Rickettsial Rocky Mountain spotted fever
2	Neoplasms	Monocytic leukemia Hodgkin s disease reticuloendotheliosis
3	During recovery from infections and agranulocytosis polycythemia vera	

Table 14 Causes of Eosinophilia

1	Allergic reactions	Bronchial asthma drug reactions allergic dermatitis hay fever angioneurotic edema
2	Parasitic infestation	Intestinal (hookworm tapeworm ascaris Taenia echinococcus) especially with muscle invasion by trichina
3	Skin diseases	Exfoliative dermatitis pemphigus dermatitis herpetiformis
4	Neoplasms	Metastatic carcinoma myelocytic leukemia Hodgkin s disease
5	Other diseases	Periarteritis nodosa eosinophilic granuloma

A decrease in the number of circulating leukocytes which is generally due to a decrease in the granulocytes is found in a variety of clinical conditions The most frequent causes of neutropenia are listed in Table 15 Although the factors that may produce neutropenia are not as well understood as those that produce anemia the possible mechanisms can be classified in a similar fashion (1) diminished production (2) increased destruction and (3) the "loss" of leukocytes from the circulation by sequestration in the various organs or tissues In the majority of clinical conditions with neutropenia diminished production appears to be the important mechanism

Diminished production may be due to a deficiency of essential substances such as vitamin B_{12} and folic acid or to the presence of metabolic antagonists such as aminopterin and 6 mercaptopurine Diminished granulocytopoiesis may also be the result of damage to the precursor cells in the marrow by irradiation chemicals marrow replacement drug idiosyncrasy and other unknown causes

The importance of leukocyte antibodies as a mechanism responsible for the production of neutropenia is uncertain The occurrence of nonspecific transfusion reactions characterized by chills fever and leukopenia associated

with the presence in the recipient's blood of agglutinins against the donor leukocytes has been reported [2] In another study it was found that leuko agglutinins were demonstrable in 38 of 350 patients with hematologic dis orders However 64 per cent of the patients with leukoagglutinins had no leukopenia and only one of 22 patients with leukopenia had leukoagglutinins Ninety per cent of the patients with leukoagglutinins had received trans fusions and the likelihood of a positive test was greater after multiple trans fusions Because of this observation and the failure to demonstrate any agglutination of autologous leukocytes it was concluded that at least the majority of leukoagglutinins are isoagglutinins [6] A different experience is reported by Tullis who employed a different technique [3] He found positive tests for leukocyte antibodies in 59 of 132 sera from patients with leukopenia and in only 11 of 102 sera from patients with hematologic and hepatic dis orders without leukopenia Antibodies were demonstrated in patients with *primary agranulocytosis primary hypersplenism secondary hypersplenism* myelophthisic anemia leukemia and drug sensitivity The anti leukocyte antibody activity which appeared to be made up of two components de creased as clinical improvement occurred and the granulocyte counts rose after treatment with steroids or splenectomy

Splenomegaly from diverse causes such as portal hypertension tubercu losis kala azar and involvement by diseases of the lymphoma group may be associated with a neutropenia that is relieved by splenectomy Whether splenectomy removes an organ of leukocyte destruction or removes a site of the production of leukocyte antibodies or eliminates a depressive influ ence on the bone marrow is uncertain

The neutropenia that occurs in anaphylactic shock and after the admin istration of histamine and nicotinic acid is thought to be due to a loss of the leukocytes from the circulation as a result of sequestration in the tissues of the body particularly the splanchnic circulation liver spleen and lungs

Table 15 Causes of Neutropenia

1 Infections Acute viral (rubeola infectious hepatitis) bacterial (brucellosis typhoid fever) protozoal (malaria kala azar) and overwhelming infections (septicemia)

2 Bone marrow damage Aplastic anemia or neutropenia due to unknown cause irradia tion toxic drugs and chemicals (benzol mustard drugs antimetabolic agents) drug idiosyncrasy (amidopyrine sulfonamides and others)

3 Disorders associated with splenomegaly Congestive splenomegaly diseases of the reticuloendothelial system (parasitic diseases chronic infections neo plasms) Felty's syndrome

4 Other disorders Disseminated lupus erythematosus anaphylaxis aleukemic" leu kemia

5 Nutritional deficiency vitamin B_{12} folic acid

AGRANULOCYTOSIS

Synonyms

Agranulocytic angina granulocytopenia malignant neutropenia primary granulocytopenia

Historical Introduction

Although isolated cases of this disease had been seen prior to that time it was Schultz's report in 1922 that established agranulocytosis as an entity[6] This report described five fatal cases that occurred in women whose illness was characterized by necrotic ulceration of the oropharynx accompanied by marked leukopenia and granulocytopenia After this description additional instances of the same disorder which was thought to be the result of some infectious agent were soon recognized However Roberts and Kracke established that the sepsis which occurred in nearly all patients with the disorder followed the agranulocytosis and was not a causative factor[4] A further advance in the knowledge of the disease came when Kracke noted that the disease occurred largely in Germany and the United States particularly in middle aged women who gave a history of treatment with drugs He also noted that the coal tar derivatives had come into wide usage in these countries following World War I The fact that benzene is a leukocyte depressant led him to suspect that benzene or its products should be seriously considered as the cause of the condition a belief that was strengthened when he was able to produce the hematologic and clinical features of agranulocytosis in rabbits by the subcutaneous injection of benzene and its oxidation products[44, 4]

Madison and Squier[48] Plum and others[43] established that drugs containing amidopyrine were responsible for most cases of agranulocytosis at that time In 1935 Kracke and Parker noted that of 172 cases that had been reported in the literature 153 had occurred after the administration of amidopyrine[43] In the same report they listed 46 American proprietary compounds that contained amidopyrine Because the number of reported instances of agranulocytosis was small in comparison to the large number of persons who took amidopyrine in one compound or another an idiosyncrasy was suspected This was demonstrated conclusively by Madison and Squier in 1934[48] and by Plum in 1935 It was recognized that agranulocytosis could be caused by other drugs and in 1938 Fitz Hugh listed 14 drugs thought to be responsible[41] This list was compiled at the beginning of the sulfonamide era and since then the list has lengthened steadily

Mechanism of the Disease

Agranulocytosis was considered to be an infectious disease until it was demonstrated that the primary factor was the reduction of the granulocytes in the peripheral blood which lowered resistance and led to bacterial invasion[44, 4] The drugs that were the most common offenders contained a known leukocyte depressant in the form of the benzene ring, and it was known that the marrow of some fatal cases was almost completely devoid of cells of the granulocytic series Because of these observations it was thought that the responsible drugs or their breakdown products acted by direct suppression of granulocytopoiesis in the marrow[43, 44, 4, 54] The greater

likelihood of a different mechanism was shown by Madison and Squier[48] They administered small doses of amidopyrine to two patients who had recovered from agranulocytosis caused by amidopyrine symptoms developed in two or three hours and the circulating granulocytes diminished greatly or disappeared completely by the twelfth hour recovery occurred in four days These observations which were confirmed by others[38] indicated that some mechanism other than depression of granulocytopoiesis must have acted to produce so profound a change in such a short time

A possible explanation of the rapid disappearance of the circulating granulocytes in these circumstances was provided by the experiments of Moeschlin and Wagner [0] Blood was obtained from a Pyramidon sensitive patient three hours after the administration of 0 3 gm Pyramidon When 300 ml of this blood was given to two normal recipients severe granulocytopenia developed in from twenty to forty minutes and persisted for three or four hours The same workers also demonstrated that at the height of the Pyramidon agranulocytosis the plasma and serum contained a substance that caused agglutination of both homologous and heterologous leukocytes It was suggested that leukocytes agglutinated in this manner were removed from the circulation and destroyed mainly in the lungs The existence of such a mechanism was demonstrated in guinea pigs which were injected with the anti leukocyte serum produced by the injection of guinea pig leukocytes into rabbits[49]

Although the presence of leukocyte antibodies may explain the occurrence of agranulocytosis in certain hypersensitive patients it appears that this mechanism is not the only one concerned in all instances of agranulocytosis that occur as a result of drug idiosyncrasy Amidopyrine appears to produce agranulocytosis by means of a true immunologic reaction that may follow the administration of only a very small amount of the drug However the agranulocytosis that sometimes follows the administration of sulfonamide compounds probably is the result of another mechanism because agranulocytosis is rarely seen during therapy with these drugs unless a fairly large amount of the drug has been administered

The role that the bone marrow plays in the production of agranulocytosis has not been clearly defined It is probably an important one in the development of the clinical syndrome The depletion of the granulocytic series of the marrow that is seen at autopsy [4] and the delay in the development of agranulocytosis until one or two weeks after the last dose of offending drug that is seen in some patients[38, 34] appear to be explained best by the action of the responsible mechanism on all members of granulocytic series in the marrow The normal and hyperplastic marrows that have been found in some patients with the disorder have been interpreted by some as evidence of partial recovery from previous damage[39, 4] and by others as a maturation arrest[41] Still another interpretation has been offered namely that the hyperplasia of the granulocytic elements in the marrow is an effort to compensate for the destruction of leukocytes in the circulation according to this concept the picture of marrow depletion develops only when the bone marrow has

become exhausted[49, 50] This interpretation appears unlikely in view of the results that have been obtained with leukopheresis experiments

The drugs that are most likely to produce a decrease in one or more of the types of cells in the blood because of an idiosyncrasy have been listed and classified by Osgood[51] and Welch, Lewis and Kerlan[57] Osgood's classification is used in Table 16 and the additional drugs mentioned by Welch et al have been included

Table 16 Drugs That Sometimes Produce Hematologic Abnormalities (Classification and Estimated Risk)

1	Anticonvulsants	methyl phenyl-ethyl hydantoin (Mesantoin)—high trimethadione (Tridione)—moderate
2	Antihistaminics	phenothiazine type (Phenergan)—moderate ethylenediamine type (Pyribenzamine)—low
3	Antimicrobial Agents	arsenobenzol—high chloramphenicol—high sulfonamides—moderate thiosemicarbazone—moderate streptomycin—low oxytetracycline chlortetracycline
4	Antithyroid Agents	thiouracils—high methimazole (Tapazole)—moderate
5	Sedatives	Sedormid—high amidopyrine—high phenacemide—moderate pyrithyldione—lo chlorpromazine
6	Spasmolytics	phenothiazine (Diparcol)—moderate procaine amide (pronestyl)—low
7	Unclassified	gold preparations—high phenylbutazone (Butazolidin)—high nitrophenols—high mercurial diuretics—low quinidine—low quinacrine hydrochloride (Atabrine)—low mercury amphetamine—low

Clinical Manifestations

Agranulocytosis occurs in both sexes and may develop at any age Prodromal symptoms such as fatigue headache weakness insomnia and restlessness usually occur The majority of patients complain of a sore throat This often progresses rapidly as chills increasing fever and dysphagia develop Unless improvement occurs the temperature rises to 105 F or more and the patient becomes mentally confused, irrational prostrated or stuporous

Physical examination early in the disease may reveal nothing more than a few small areas of necrosis in the tonsillar areas Later the areas of necrotic ulceration enlarge as edema and slight reddening of the mucous membranes develop The cervical lymph nodes become enlarged and slightly tender and in some patients the soft tissue of the neck becomes swollen At times vaginal and rectal ulcerations occur

Laboratory Examination

The most significant feature is the occurrence in the peripheral blood of severe leukopenia and granulocytopenia unaccompanied by anemia or thrombocytopenia Many patients develop lymphocytopenia as well as granulocytopenia and the total leukocyte count falls to levels as low as 100 to 2000

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per cent[39] The mortality rate was nearly 100 per cent in patients whose leukocyte counts fell to less than 1000 per cu mm and in persons over 60 years of age The general condition of the patient and the amount of drug that had been administered also appeared to influence the outcome[39]

In the pre-antibiotic era many forms of treatment were employed in an effort to produce an increase in granulocytes These included x ray therapy blood transfusion injections of pentose nucleotide leukocyte concentrates extracts of liver or bone marrow The early reports on each method were hopeful but subsequent experience was disappointing The first real advance in treatment came when Dameshek and Wolfson administered sulfathiazole a drug known to be capable of producing agranulocytosis to two moribund patients whose disease was due to some other drug the therapy appeared to bring the infection under control and the patients recovered[40]

The most important aspects of treatment are the withdrawal of the offending drug and the administration of a chemotherapeutic agent to combat infection The daily administration of 300 000 units of procaine penicillin and 0 5 gm streptomycin has been recommended for moderately severe cases[51] if the disease is severe or progressive this dose is given twice daily and is supplemented with penicillin G 500 000 units daily if symptoms progress It is recommended that treatment be continued for a week after the lesions heal[51] The use of penicillin alone has been recommended (600 000 to 1 200 000 units daily) tetracycline 0 5 gm every six hours or some other wide spectrum antibiotic is added if necessary[46]

Illustrative Case

This 62 year old white widow was in good health until six weeks prior to admission when she developed hematuria frequency of urination dysuria and suprapubic pain A diagnosis of urinary tract infection was made and she was treated with a sulfonamide (Sulamyd) 0 5 gm three times a day The urinary symptoms improved and the medication was continued for about five weeks At this time the patient complained of fatigue lassitude and chilliness She was admitted to her community hospital where she was found to have a leukocyte count of 600 per cu mm and a temperature of 101 F She was treated with penicillin transfusions and cortisone Four days later the leukocyte count had risen to 2000 per cu mm but granulocytes were still absent from the peripheral blood and she was referred for further treatment.

Physical Examination Temperature 103 pulse 84 respirations 22 There was no ulceration of the nasopharynx or vagina and the liver spleen and lymph nodes were not enlarged The remainder of the examination was noncontributory

Laboratory Examination Blood studies on admission hct 55 per cent wbc. 2000 per cu mm differential count (per cent) juveniles 1 bands 6 segmented 9 lymphocytes 70 monocytes 12 basophils 2 reticulocytes 1 3 per cent platelet count normal Bone marrow obtained by aspiration five

per cu mm Granulocytes often constitute less than 10 per cent of the total cells and may be entirely absent Those that are present often show extreme degrees of toxic granulation Monocytes often make up an appreciable percentage of the cells especially during recovery and a total monocyte count of over 100 per cu mm is considered a sign of a good prognosis [3, 5]

The appearance of the bone marrow varies with the stage of the disease *In most of the severe cases at the height of the disease the cells of the granulocytic series are greatly reduced or absent and the percentages of lymphocytes plasma cells and reticuloendothelial cells are increased* [39, 4] In other patients particularly when recovery is taking place examination of the marrow reveals hyperplasia of the granulocytic series in such circumstances toxic granulation is evident in many of the younger cells [39, 44, 47]

In severe cases blood cultures often establish the presence of septicemia. Smears made from the ulcerated lesions or sputum often show masses of bacteria accompanied by but few cells

Differential Diagnosis

The most important consideration in the differential diagnosis is acute leukemia The distinction is usually not difficult because leukemia is generally associated with anemia thrombocytopenia and hemorrhagic manifestations which are not a part of agranulocytosis Examination of the bone marrow and study of smears made from the buffy coat of the peripheral blood nearly always reveal the presence of blasts and other young cells when leukemia is present A carefully taken history regarding recent exposure to drugs or chemical agents is often helpful

The presence of agranulocytosis may be suspected in some patients with infectious mononucleosis who have ulcerative pharyngeal lesions and a marked reduction in the granulocytes in the peripheral blood The degree and distribution of the adenopathy the presence of pathologic lymphocytes in the blood smear and the positive heterophil agglutination test usually lead to the recognition of infectious mononucleosis in doubtful cases a few days of observation generally resolve the difficulty because leukopenia rarely persists for more than a few days in infectious mononucleosis

Other diseases are less likely to be confused with agranulocytosis Sepsis is rarely responsible for a leukocyte count of less than 3000 per cu mm or a depression of the granulocytes to less than 30 per cent of the total Other diseases such as those of the lymphoma group tuberculosis and subacute bacterial endocarditis are sometimes associated with severe leukopenia In such patients the underlying disease is usually easily recognized because of splenomegaly or other features that lead to the correct diagnosis

Prognosis and Treatment

Before antibiotics became available agranulocytosis was a serious disease with a mortality rate in the well developed cases that varied from 50 to 90

changes in the neutrophils In addition no significant fluctuations were found in the levels of gamma globulin or properdin

In some patients treatment with corticosteroids and splenectomy appear to modify temporarily the clinical manifestations of the disease without producing a significant alteration in the cyclic neutropenia[91] Splenectomy appears to produce more lasting benefit in older patients with definite splenomegaly [8] Antibiotics are important in the treatment of infections during the neutropenic phases but their long term prophylactic use is not recommended

INFECTIOUS MONONUCLEOSIS

Synonyms

Glandular fever acute benign lymphoblastosis monocytic angina acute lymphadenosis

Historical Introduction

Credit for the first description of this disease is generally given to Pfeiffer for his report on Drusenfieber which appeared in 1889[9] Pfeiffer described an illness occurring in children five to eight years of age which was characterized by fever of one to ten days duration sore throat adenopathy splenomegaly and hepatomegaly The disorder was considered to be an infectious and epidemic disease Following this account reports of unusual cases of adenitis that were associated with marked lymphocytosis and resembled lymphocytic leukemia appeared in the literature[67] but the report of Sprunt and Evans in 1920 provided the first clear description of the disease to which they gave the name infectious mononucleosis[1] They studied six young adults whose illness was characterized by fever enlargement of the cervical axillary and inguinal lymph nodes splenomegaly and leukocytosis The differential count was unusual because of an increase in mononuclear elements chiefly large lymphocytes even more important was the observation that all types of pathological lymphocytes some resembling Turk s irritation forms were to be seen" Study of lymph nodes removed from three patients disclosed definite hyperplasia distinct from Hodgkin s disease tuberculosis and lymphosarcoma that could not be distinguished from lymphocytic leukemia It was concluded that the cases constituted a clinical syndrome that had a good prognosis In the next few years groups of similar cases were reported by Bloedorn and Houghton[5] Longcope[93] and Tidy and Morley[11] and it was generally agreed that the diseases glandular fever and infectious mononucleosis were identical

The abnormal mononuclear cells that had been noted were studied carefully by Downey and McKinlay in 1923[3] Their observations have been confirmed many times but little has been added to their description In a study of nine cases they recognized three types of abnormal lymphocytes in the blood and concluded that there was no danger of confusing the blood picture with leukemia if well studied"

An important contribution that facilitated the recognition of the disease

days after the agranulocytosis was recognized was cellular erythropoiesis was normoblastic in type and the megakaryocytes were normal The cells of the granulocytic series were increased Differential count of the marrow leukocytes (per cent) promyelocytes 0 5 myelocytes 16 juveniles 14 bands 46 segmented cells 18 lymphocytes 5 eosinophils 1 The patient was treated with penicillin 500 000 units per day Her temperature returned to normal by the third day and remained normal thereafter The leukocyte count rose rapidly On the day after admission it was 3500, the third day 10 000 the sixth day 18 000 the fourteenth day 21 000 One month after discharge from the hospital and about seven weeks after onset of symptoms of agranulo cytosis her hematocrit was 47 per cent hemoglobin 15 gm per 100 ml leukocyte count 5000 per cu mm, platelets 230 000 per cu mm The differen tial count was normal

Comment This elderly patient had agranulocytosis manifested by severe leukopenia associated with normal red cell and platelet counts The bone marrow specimen which was examined after recovery had begun ap peared hyperplastic That obtained at another hospital a week earlier was either aplastic or dilute The extremely low leukocyte count in this patient would have augured a poor prognosis in the days before antibiotics On treatment with penicillin and supportive measures she made an uneventful recovery When agranulocytosis is caused by a sulfonamide drug which was suspected but not proved in this case the fall in granulocytes usually does not occur until after the patient has been taking the drug for several weeks The patient was warned against future use of the suspected drug

CYCLIC NEUTROPENIA

Cyclic neutropenia is a well established but poorly understood entity that was first described by Leale in 1910 [59] Recent reviewers have found 30 cases reported in the literature [8, 60] This rare disorder which may occur at any age is characterized by the regular diminution or disappearance of the neutrophilic granulocytes from the circulation at approximately 21 day in tervals During the neutropenic phase fever malaise and oral pharyngeal ulceration usually appear and are often accompanied by arthralgia head *ache sore throat and lymph node enlargement Relatively minor intercur* rent infections occur commonly but severe infections are unusual [8]

The careful studies of Page and Good have provided the clearest picture of the disorder [61] By serial bone marrow studies they showed that the recur rent neutropenia is due to the cyclic arrest of production of the entire neu trophilic series Neither a neutropenia producing factor nor leukoagglutinins could be demonstrated in the blood of the patient The patient s serum ex erted no demonstrable influence on *the motility function of normal neutro* phils Although the influence of a hormonal factor could not be excluded none was demonstrated The occurrence of neutropenia bore no relationship to menstruation or to the urinary excretion of FSH or 17 ketosteroids The concentrations of corticotropin and 17 hydroxycorticosteroid in the blood and the response of the adrenal gland to stimulation by corticotropin re mained within normal limits without fluctuation throughout the cyclic

was made by Paul and Bunnell in 1932.[96] These investigators utilized a sheep cell agglutination test to study the presence of heterophil antibodies in patients with rheumatic fever and in a group of individuals who served as controls. In their report they stated, "Quite by accident it was discovered that heterophil antibodies were present in a specimen of serum from a patient ill with infectious mononucleosis in much higher concentration than has been described in serum disease or any other clinical condition that we studied." They obtained similar results in three other patients with infectious mononucleosis and, apart from serum sickness and one other exception, failed to find high titers of heterophil antibodies in a large number of other conditions. Since that time the heterophil agglutination test has proved helpful in diagnosing infectious mononucleosis. However, an increasing number of positive tests have since been found in conditions other than infectious mononucleosis and for this reason Davidsohn's differential test has proved valuable.

Comprehensive monographs on infectious mononucleosis have been written by Bernstein[63] and Leibowitz,[90] and Tidy has given an excellent review of the history and clinical features of the disease.[111]

Mechanism of Disease

The cause of infectious mononucleosis is unknown. It is generally thought to be an infection,[63, 80, 111, 112] probably viral in origin, even though efforts to isolate an infectious agent or transmit the disease to human volunteers by the use of nasal washings, lymph node suspension, plasma, whole blood, and serum have been unsuccessful, with one possible exception.[118] The disease, which appears to have a low degree of infectivity, occurs sporadically and in epidemics.[80, 110, 116] The incubation period has been estimated at five to fifteen days,[111] one to twenty-eight days,[63] four to eighteen days,[80] and five to eight weeks.[8]

Most of the clinical manifestations of the disease are explained by the pathologic findings. The most complete report is that of Custer and Smith.[71] In a study of nine autopsies they found that the lymph nodes were usually enlarged as a result of an increase in the size of the lymph follicles. Enlargement of the lymph nodes was not invariable and in some cases the architecture of the nodes was blurred and the follicles were inconspicuous. Splenic enlargement occurred in every instance and was associated with lymphocytic infiltration of the trabeculae and capsule. Varying degrees of lymphocytic infiltration were also found in the tissue of the central nervous system, meninges, myocardium, and in the periportal areas of the liver. In this series the bone marrows were normal or hyperplastic. In another study of 23 bone marrow biopsies granulomatous lesions were found in nine.[6]

Figure 49. Acute leukemia.

Figure 50. Infectious mononucleosis.

(From Heilmeyer, L., and Begemann, H.: Atlas der klinischen Hamatologie und Cytologie. Berlin, Gottingen, Heidelberg, Springer, 1955.)

and was characterized by a temperature of 101 to 103 F moderate glandular enlargement and frequent recrudescence In the "anginose" type the prodromal period lasted from one to three weeks following which the patient developed a severe membranous sore throat clinically indistinguishable from diphtheria, peritonsillar edema and a temperature of 104 F The "febrile" type was characterized by the sudden onset of malaise headache chills and temperature of 102 to 103 F a rash was often noted during the first week and glandular enlargement did not appear until from ten to twenty one days had passed Other authors have classified patients on the basis of outstanding clinical features as "pharyngeal" or "typhoidal"[8] and as "anginose" "insidious" "eruptive" "icteric" "pulmonary" "abdominal" "lymphoglandular" and "meningitic"[116]

The incidence of the symptoms and signs in patients with infectious mononucleosis varies in different series[63, 8, 60, 94, 106, 10, 111, 116] The extremes are shown in Table 17

Table 17 The Incidence of Various Symptoms and Signs in Infectious Mononucleosis as Reported by Different Authors

SYMPTOMS	PER CENT	PHYSICAL SIGNS	PER CENT
Sore throat	50–80	Fever	70–100
Malaise	25–63	Enlarged nodes	76–100
Headache	35–67	Pharyngitis	68–85
Weakness	13–54	Splenomegaly	26–70
Anorexia	17–41	Hepatomegaly	6–37
Abdominal pain or disease	14–22	*Rashes*	0–18
Ocular symptoms	6–15	Jaundice	5–8
		Hematuria	1–7

Skin Lesions Various types of cutaneous eruptions occur and an incidence of 18 per cent was found in one series The rash usually makes its appearance between the fourth and seventh days[63, 111] but may appear as late as the twentieth day[1] The commonest type is a fine macular or maculopapular rash pink or pinkish brown in color that involves mainly the trunk The rash is often virtually indistinguishable from that seen in rubella The rash which may consist of only eight or ten lesions usually appears in a single crop that lasts from three to seven days[63, 10, 111] Other types of lesions that have been seen less often are erythematous urticarial morbilliform and vesicular Lesions like those seen in erythema multiforme and erythema nodosum also occur[3, 5, 6, 1]

Liver Damage Although jaundice occurs in only about 6 per cent of patients with infectious mononucleosis it is probable that *some impairment* of liver function occurs in a high percentage of patients with the disease Study of liver tissue obtained by biopsy and at autopsy indicates that the jaundice is due to a form of hepatitis and is not the result of extrahepatic obstruction from enlarged nodes[1, 9, 84, 86] The lesion consists mainly of periportal infiltration with mononuclear cells there are minimal changes in the hepatic cells which are not accompanied by necrosis or alteration of the

Clinical Manifestations

Infectious mononucleosis occurs in both sexes and is predominantly a disease of children and young adults The disease has been reported in a four month old infant and in an adult seventy years of age[111] About 80 per cent of the cases occur between the ages of 15 and 30[63, 80, 87] and the disease is rarely seen in persons over 40[111] At one time infectious mononucleosis was considered rare in Negroes[63] but a more recent report indicates that the incidence in Negroes is about the same as that in Caucasians[116]

Although infectious mononucleosis varies a great deal in its manner of onset severity and clinical manifestations the majority of patients have a similar clinical picture The patient with infectious mononucleosis usually *complains of malaise sore throat headache and weakness of several days* duration Anorexia and cough are not unusual Fever of less than 102 F is usually present and the pulse is generally normal or slow in proportion to the temperature Examination of the pharynx usually reveals diffuse injection of the mucous membranes or follicular tonsillitis Palpable enlargement of the cervical lymph nodes is usually evident when the patient is first seen or develops within a few days Involvement of the nodes in the cervical region which is generally bilateral is soon followed by the appearance of enlargement of the axillary and inguinal nodes The spleen and liver are often palpable Usually both the characteristic findings in the blood a lymphocytosis associated with pathologic lymphocytes and the positive heterophil agglutination test are present when the patient is first seen or develop during the first week or ten days thereafter As a rule the fever *subsides by lysis within the first or second week and the symptoms disappear* during the second or third week Convalescence is rapid in most patients The glandular enlargement often improves within a few days but some residual enlargement may persist for months In three fourths of the patients the blood picture returns to normal in two months and the heterophil agglutination becomes negative in less than four months[111]

The "usual" clinical picture is not seen in a number of patients with *infectious mononucleosis Some patients are essentially asymptomatic* and the presence of the disease is manifested only by painless adenopathy and the characteristic laboratory findings Other patients become *acutely ill with* high fever and present the appearance of a severe overwhelming infection In still other patients the clinical features are dominated by the presence of the disease in a particular organ in such patients the presenting complaints are abdominal pain jaundice hematuria or disease of the central nervous system.

The essential features of infectious mononucleosis as emphasized by Tidy are fever glandular enlargement, faucial infection and lymphocytosis[111] The prominence of these features varies in different patients and various authors have recognized different groups of *clinical syndromes* Tidy classified his patients into "glandular" "anginose" and "febrile" types The "glandular" type occurred most often in children five to eleven years of age

present in the autopsied cases Although the electrocardiographic changes persist for months in some patients[103] there is little or no evidence that chronic heart disease occurs in patients with infectious mononucleosis

Rupture of the Spleen This is a serious complication that has been seen in at least 21 patients with infectious mononucleosis[10] This complication which has a mortality rate of about 33 per cent in the reported cases[10] is one of the most frequent causes of death in infectious mononucleosis[71] Rupture of the spleen occurs as a consequence of splenic enlargement associated with capsular infiltration and subcapsular hemorrhage[71 88 101 103] The rupture rarely occurs before the third week of the disease but has been reported as early as the end of the second week[68 101] and as late as thirty days after onset[101] The presence of this complication is generally manifested by severe pain in the upper abdomen or left upper quadrant followed by circulatory collapse In some patients the occurrence of this complication has not been recognized because the diagnosis of infectious mononucleosis had not been made prior to the rupture and was not suspected then because the intraperitoneal hemorrhage was associated with polymorphonuclear leukocytosis[101] Although rupture of the spleen in infectious mononucleosis is usually termed "spontaneous" the importance of slight trauma associated with a trivial blow or the performance of the physical examination has been emphasized[101 113]

Hematologic Complications The most important hematologic complications that occur in infectious mononucleosis are hemolytic anemia and thrombocytopenic purpura Both complications are rare but each has been reported at least 16 times[31] In the reported cases the hemoglobin levels have ranged from 4 3 to 9 8 gm per 100 ml the reticulocyte count from 7 0 to 37 5 per cent and the level of indirect reacting bilirubin in the serum from 1 6 to 6 0 mg per 100 ml[109] The antiglobulin (Coombs) test has been positive in most of the patients with hemolytic anemia when it has been performed[16 109] which suggests that the hyperhemolysis is due to autoimmune antibodies Both the anemia and the thrombocytopenia disappear spontaneously or with appropriate treatment and no deaths have been reported from these complications

Hematuria Hematuria occurs in from 3 to 6 per cent of patients[111 116] and may be the presenting complaint[115] Presumably the hematuria results from lymphocytic infiltration in the kidney[66 1] Chronic renal disease secondary to infectious mononucleosis has not been reported

Laboratory Manifestations

The most characteristic abnormalities in the peripheral blood in infectious mononucleosis are leukocytosis absolute lymphocytosis and the presence of abnormal lymphocytes Leukocyte counts of 5000 per cu mm or less may occur early in the disease[63] but the total count is usually elevated by the second week[63 90 94] Counts over 40 000 per cu mm are rare[63] The most constant feature in infectious mononucleosis is the presence of the

hepatic architecture A high incidence of abnormal liver function tests has been found in patients with *infectious mononucleosis without jaundice*[69, 70, 6, 98, 116] In one series of 15 patients the thymol turbidity and BSP tests were found to be positive in all and the alkaline phosphatase level was elevated in 7 of 8 patients on whom it was determined Plasma electrophoresis was performed on 6 patients and a protein distribution similar to *that seen in infectious hepatitis was found* there was a slight decrease in albumin increase in alpha and beta globulin and a marked increase in gamma globulin Other studies have shown a positive thymol turbidity test in 60 to 68 per cent[69, 6, 116] and a positive cephalin flocculation test in 95 per cent [6] Such studies have led to the conclusion that hepatitis is a feature of nearly all cases of infectious mononucleosis [90] Although abnormal liver function tests may persist for long periods (up to 22 months in one report) [116] the prognosis for complete recovery is excellent even in the patients with jaundice Cirrhosis of the liver has been reported only once and this patient had an alcoholic history [89]

Central Nervous System Involvement Involvement of the central nervous system first reported in 1931 [4, 83] is a rare complication of infectious mononucleosis Up to 1955 64 cases had been reported [10] Involvement of the central nervous system has been manifested by headache nuchal rigidity drowsiness stupor coma paralysis convulsions and changes in the spinal fluid such as pleocytosis increased protein and increased pressure The types of central nervous system involvement were classified by Bernstein and Wolff as follows (1) serous meningitis (2) meningitis (3) encephalitis (4) meningo encephalitis (5) meningitis or meningo encephalitis with acute polyneuritis (6) peripheral neuropathy [64] Meningitis encephalitis and meningitis or meningo encephalitis with polyneuritis (Guillain Barre syndrome) make up about three fourths of the reported cases [10] Although central nervous system involvement is estimated to occur in less than one per cent of the patients with infectious mononucleosis [64] this complication is responsible for a high percentage of deaths in the disease when death occurs it is usually the result of respiratory paralysis [1] Despite a course that *is frequently stormy the prognosis is good in patients with central nervous* system involvement 85 per cent recover within a period that varies from a few days to several months after the onset of the complication [64]

Cardiac Involvement The occurrence of cardiac involvement has been reviewed [81] There is rarely any clinical evidence of cardiac involvement but pericarditis which was diagnosed by the occurrence of chest pain pericardial friction rub electrocardiographic abnormalities and changes in heart size has been reported nine times [7, 94, 10, 103] Most of the reported cardiac abnormalities are not obvious and consist of electrocardiographic changes in the T waves P R interval or cardiac rhythm [81, 10, 116] Routine electrocardiograms have revealed abnormalities in from 5 to 50 per cent of patients [81, 116] Postmortem studies have revealed focal interstitial infiltration of abnormal lymphocytes in the myocardium that appears to offer an explanation for the electrocardiographic findings [1] Pericarditis has not been

rickettsialpox, and allergic states[9] In these disorders the percentage of abnormal lymphocytes is generally less than in infectious mononucleosis and elevated total leukocyte counts are unusual Although absolute lymphocytosis nearly always occurs in infectious mononucleosis it is not a constant finding An increase in polymorphonuclear cells sometimes occurs in the prodromal phase and early in the disease[5, 112]

Eosinophilia which is more likely to develop during convalescence[90, 116] has been found in 14 per cent of one series[98] and in 10 per cent of another[116] The eosinophils are usually less than 15 per cent but counts as high as 21 per cent occur Except in rare instances complicated by hemolytic anemia and thrombocytopenia the hemoglobin red cell count, and platelet counts are normal

Although the heterophil agglutination (Paul Bunnell) test is nonspecific it is a very helpful procedure in the diagnosis of infectious mononucleosis[96] Titers of 1 56[98] 1 160[3] and 1 224 have been considered confirmatory of infectious mononucleosis if the clinical and hematologic findings are suggestive of the disease The differential adsorption test of Davidsohn is more specific because agglutinins for sheep cells sometimes occur in normal persons and in patients with serum sickness and other diseases[2] Davidsohn found that the heterophil antibodies in infectious mononucleosis are not completely removed from the serum by adsorption with guinea pig kidney but are removed completely when adsorbed with beef red cells The heterophil antibodies found in normal individuals and in patients with serum sickness and other diseases are different in that they are removed from the serum when adsorbed with guinea pig kidney The test is useful in excluding patients with high titers that are not due to infectious mononucleosis and in establishing the diagnosis of infectious mononucleosis in patients with low titers The test has been particularly helpful in patients with monocytic leukemia and acute leukemia titers as high as 1 896 have been seen in these diseases but they could be distinguished from infectious mononucleosis by the guinea pig absorption test[9, 3, 4] The incidence of positive heterophil agglutination tests in infectious mononucleosis has been reported to be from 50 per cent to 100 per cent but is probably around 70 per cent[12, 90, 91] The frequency of positive tests depends on the criteria used for establishing the diagnosis of infectious mononucleosis the stage of the disease and the number of the tests performed The test is usually positive when the patient is first seen[0, 8, 9] but the time of development of a positive test varies from one to 89 days In the vast majority of patients the test will be positive in the first two weeks of the disease if it is going to become positive[80] The heterophil agglutination test may remain positive from 9 days to 63 weeks[8] but generally it becomes negative in from two to four months[83] In one series the test became negative in 75 per cent of the patients within four months

The thymol turbidity BSP excretion and cephalin flocculation tests and the serum alkaline phosphatase level have been found to be abnormal in a high percentage of patients with infectious mononucleosis[79, 9, 6, 89] One

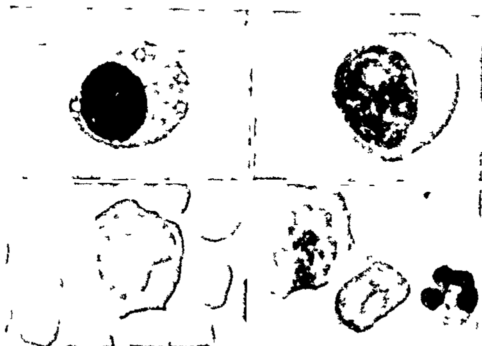


Figure 51 Various forms of pathologic lymphocytes seen in patients with infectious mononucleosis

pathologic lymphocytes" first described by Sprunt and Evans[10] and classified into three types by Downey and McKinlay [3] The Type I cell is a highly differentiated mature lymphocyte with a nucleus that has a cloudy appearance due to the presence of a coarse network of chromatin strands or masses that is not sharply separated from the parachromatin The nucleus is often indented The cytoplasm is very basophilic and somewhat mottled in appearance The Type II cell has a nucleus with a coarse chromatin structure somewhat similar to that of the plasma cell The cytoplasm is less basophilic than the Type I cell and contains fewer vacuoles The Type III cell which more closely resembles leukemic cells has a less differentiated nucleus that contains nucleoli In Downey and McKinlay s report the Type III cells were uncommon they were seen in small numbers in only one of nine cases while the Type I and II cells were numerous in all nine cases that were studied

Other abnormalities such as nuclear indentation cytoplasmic vacuolation and nuclear fenestration may be seen in lymphocytes of all sizes In patients who are diagnosed as having infectious mononucleosis mononuclear cells almost invariably constitute over 50 per cent of the leukocytes at some stage of the disease [63 90 98 111] and counts as high as 96 per cent have been observed [63] Abnormal lymphocytes probably occur in all patients with infectious mononucleosis but are not pathognomonic of the disease Apparently identical cells for which the name "virocyte" has been suggested have been observed in the following conditions infectious hepatitis virus pneumonia herpetic infections rubella rubeola roseola infantum undulant fever

nopathy or evidence of central nervous system involvement is the presenting complaint the diagnosis may not be considered in sporadic cases when the patient is first seen When the diagnosis of infectious mononucleosis is considered repeated examination of the peripheral blood repeated agglutination tests and a period of observation may be necessary before the question can be settled

Course and Prognosis

Infectious mononucleosis usually runs an acute course characterized by fever for ten days or less [8] followed by prompt recovery A recrudescence of fever and symptoms is not unusual after the temperature has returned to normal for several days In some patients the manifestations of the disease are so mild that the presence of the disease is recognized only by the laboratory tests In other patients malaise and weakness may persist for months before complete recovery occurs Nearly all patients recover completely but deaths have occurred in some patients with splenic rupture and in some with central nervous system complications

Treatment

The treatment of infectious mononucleosis is largely symptomatic and supportive Parenteral fluids may be necessary if the throat is much involved Prolonged bed rest is not necessary in patients with abnormal liver function tests or abnormal electrocardiograms Treatment with sulfonamides penicillin chlortetracycline and chloramphenicol has not been found to shorten significantly the duration of symptoms or fever or to reduce the incidence of complications [61 88 99 114]

Illustrative Case

A 17 year old white male student was in excellent health until two weeks prior to admission when he first noticed malaise Ten days prior to admission transient chilly sensations occurred Five days before admission severe headache appeared and was accompanied by sore throat and enlargement of the cervical lymph nodes The sore throat became progressively worse and on the day of admission the patient was unable to swallow liquids

Physical Examination The oral temperature on admission was 100 F The tonsillar areas were covered with a white thick pseudomembranous type of exudate There was bilateral enlargement of the cervical and axillary lymph nodes which measured 1 to 3 cm in diameter A soft spleen edge was palpable 1 cm below the left costal margin The neurologic examination and the remainder of the routine examination were within normal limits

Laboratory Examination Admission blood studies hct 50 per cent wbc 14 000 per cu mm differential count (per cent) granulocytes 28 lymphocytes 64 monocytes 8 Many of the lymphocytes appeared abnormal

review of the literature reports that the various liver function tests were abnormal in from 40 to 100 per cent of 524 cases[63]

The occurrence of false positive Wassermann and Kahn tests in infectious mononucleosis is well known Incidences of 2 per cent[106] 3 per cent,[116] 10 per cent [8] 18 per cent[73] and "about half"[111] have been reported The test usually becomes positive during the second week and reverts to negative by the eighth week[111] but the duration of positivity varies from a few days[63] to more than three months The finding of a positive routine serologic test for syphilis in a young adult may be the first intimation that the patient may have infectious mononucleosis

False positive agglutination tests for Br melitensis S typhosa S paratyphi A and B and Proteus X 19 have been reported[6, 87] In some patients several agglutination tests may be positive at the same time[6, 88] When *repeated titrations have been done it has been noted that the titers rose* abruptly sometimes to as high as 1 1280 but fell quickly

Transient urinary abnormalities in the form of red blood cells white blood cells albuminuria and casts have been reported in 3 per cent and 6 per cent of patients[111]

Diagnosis

The possibility of infectious mononucleosis is suggested by the occurrence of sore throat fever enlarged lymph nodes or lymphocytosis in children and young adults The presence of more than 50 per cent lymphocytes and more than 10 per cent pathologic lymphocytes is even stronger evidence Most authors accept a diagnosis of infectious mononucleosis if the clinical and hematologic picture is considered characteristic even if the heterophil agglutination test is negative[63, 90, 94, 98] However some consider that patients with a negative heterophil agglutination reaction who have the clinical and hematologic features of infectious mononucleosis really have a different disease that is closely allied to infectious mononucleosis[50, 100] The actual incidence of positive heterophil agglutination tests is hard to determine because it depends on many factors including the stage of disease when the test is performed the number of the tests done and the criteria that are accepted for the diagnosis of infectious mononucleosis Titers of over 1 56[98] and over 1 224 [2] have been considered diagnostic if the other features of the disease are consistent It has been recommended that the differential adsorption test be used if the titer is less than 1 224 and the findings suggest infectious mononucleosis and also in circumstances when the titer is over 1 224 but is associated with no evidence of the disease[7] If the titer is reduced more than 75 per cent by adsorption with guinea pig kidney the antibody is probably not of the infectious mononucleosis type

The diagnosis of infectious mononucleosis may be difficult if the patient is seen before the characteristic hematologic and serologic findings appear especially when the clinical features are those that are more often associated with other diseases When jaundice hematuria a septic type of fever ade

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AGRANULOCYTOSIS

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they were large and had an opaque basophilic cytoplasm The nuclei of many of the smaller cells were indented or fenestrated Other examinations showed the following serum bilirubin level 0 7 mg per 100 ml alkaline phosphatase 6 3 (B U) thymol turbidity 9 6 units cephalin flocculation 4 plus in 48 hours spinal fluid normal The heterophil agglutination test was positive in dilutions of 1 3564 before adsorption and positive in a dilution of 1 792 after differential adsorption

A diagnosis of infectious mononucleosis was made The patient responded well to symptomatic measures and was essentially asymptomatic when discharged from the hospital eight days later One month after discharge nodes that measured 1 × 1 cm were still palpable in the cervical area and in the left axilla The leukocyte count was 7000 per cu mm and the differential count was (per cent) bands 1 segmented 47 lymphocytes 40 monocytes 9 eosinophils 2 basophils 1 A few atypical lymphocytes persisted The heterophil test was still positive in dilutions of 1 24 after adsorption

Comment This patient illustrates most of the typical features of infectious mononucleosis The majority of patients are young adults who complain of fever malaise sore throat and cervical adenopathy As often happens the heterophil agglutination test was positive and the blood smear was typical of infectious mononucleosis when the patient was first seen The patient is typical of many with the disease in that he had neither clinical jaundice nor elevation of the serum bilirubin but did exhibit evidence of liver dysfunction in the various tests Because of his severe headache the complication of a meningo-encephalitis was considered but was discarded when the neurologic examination and cerebrospinal fluid proved to be normal

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CHAPTER IX

Agents Used in the Treatment of Leukemia and Malignant Lymphoma

THE PRINCIPAL agents used in the treatment of leukemia and lymphoma are ionizing radiation, alkylating agents, antimetabolites and the adrenocortical hormones. Each of these agents may produce serious side effects and none is curative, yet they often induce hematologic and clinical remission when employed properly.

IRRADIATION

Ionizing radiation may be administered from an external source by use of roentgen rays or cobalt^{60} or from an internal source by the administration of a radioactive isotope such as radioactive phosphorus (P^{32}). The effects produced by the irradiation of tissue cells have been studied extensively but the site of the primary biochemical lesion has not been established. A variety of enzymatic changes have been demonstrated in the cell after irradiation but

these occur late and may be secondary to some disturbance in cellular metabolism.[7, 8, 13] Inhibition of the synthesis of both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) has been demonstrated, but protein synthesis by the cell seems unaffected.[1, 6] It has been demonstrated that small doses of radiation produce a delay in the onset of DNA synthesis[43] but do not inhibit DNA synthesis once it has been initiated. At the present time it is not known whether these changes represent a primary effect of ionizing radiation on the biochemical sequence leading to nucleic acid synthesis or an effect that is secondary to mitotic inhibition.[1, 36] It has been shown that main polynucleotide chains of DNA are broken by the direct action of ionizing radiations. If this effect is relatively unselective, as it appears to be, any cellular function in which DNA is involved may be affected. It has been postulated that the primary effect of irradiation on the cell may be so small as to be undetectable at present. As the result of the initial disturbance the metabolic activities of the cell may function incompletely and produce the biochemical and anatomic changes which can be demonstrated.[2]

Because ionizing radiation interferes with deoxyribonucleic acid synthesis, it produces damage in any tissue composed of dividing cells. The damage is most apparent in the tissues with active cellular proliferation. Therapeutic irradiation aims at destroying the rapidly proliferating tumor cells while sparing the normal cells of the body as far as possible. Ionizing radiation from an external source is directed in such a manner that the amount of normal tissue in its path is as small as possible. This is accomplished by careful positioning of the patient and the use of protective shielding. The use of multiple portals of entry for the ionizing radiation when a large dose is required in the body cavity reduces the effects on the normal skin and other tissues which must be traversed to reach the lesion. The cells of the hematopoietic system, the gastrointestinal tract, and the skin, which have a high rate of turnover, are particularly susceptible to radiation damage. Damage to the cutaneous cells and depression of the bone marrow frequently limit the amount of radiation the patient can receive.

Ionizing radiation has been used extensively in the treatment of diseases of the lymphoma group and the chronic forms of leukemia but is not employed in the treatment of the acute forms of leukemia, since its use often results in a more rapid course. It was used first for the treatment of Hodgkin's disease between 1902 and 1905.[47] Since that time it has been used more than any other form of treatment for all of the diseases in the lymphoma group. It is more effective than the radioactive isotopes in the control of these disorders.[4, 4] The diseases of the lymphoma group are radiosensitive and they often undergo striking regression after exposure to a small amount of radiation. Unfortunately, the remissions are temporary and the diseases eventually become resistant to additional intensive therapy. It is customary to employ larger doses of radiation during the initial course of therapy than in the treatment of recurrences. The amount of therapy to be administered must be individually determined for each patient and depends on the nature of the disease, the region involved, the extent of disease, the condition of

the patient and the resistance of the lesion to ionizing radiation Giant follicle lymphoma is the most radiosensitive of the group and requires the smallest amount of radiation Reticulum cell sarcoma and lymphosarcoma usually require more than Hodgkin s disease Irradiation of neighboring lymphatic areas has been recommended by some on the grounds that extensions generally occur in one of the proximal lymph node regions [0] but has been considered unwise by others who feel that the prediction of further sites of involvement is impossible [46]

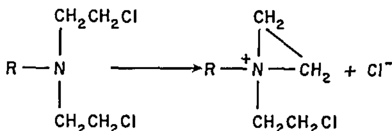
The use of radioactive phosphorus (P^{32}) which was introduced in 1936 as a source of ionizing radiation has been reviewed recently [9] This method of treatment offers certain advantages over roentgen ray therapy in that generalized irradiation of the hematopoietic system may be obtained with relative ease without the production of radiation sickness [44] P^{32} emits beta radiation which produces ionization within an area limited to its maximum range of 0 7 cm It has a 14 3 day half life and decays to stable sulfur Since the sensitivity of a cell to ionizing radiation seems related to its mitotic stage the continuous irradiation provided by P^{32} may have a distinct advantage over intermittent therapy from an external source in the treatment of a growing tissue that contains cells asynchronously undergoing cell division Following administration P^{32} is concentrated in tissues composed of rapidly metabolizing cells which have a high phosphorus turnover such as the bone marrow lymph nodes spleen and liver later the P^{32} enters the osseous tissue of the skeleton Because of the selectivity provided by its distribution within the body the chief value of radioactive phosphorus has been in the treatment of chronic leukemias and polycythemia vera

Because of the depressant effect that all ionizing radiation exerts on the bone marrow frequent leukocyte counts and hematocrit determinations are performed while the patient is undergoing therapy The level of the leukocytes that is considered a contraindication to further therapy depends on details of the individual case and no fast rules can be stated The development of leukopenia, thrombocytopenia and anemia demands caution and careful evaluation before proceeding with further therapy

ALKYLATING AGENTS

Among the most effective compounds available for the treatment of chronic leukemia and lymphoma are the alkylating agents These agents were introduced into clinical medicine as the result of extensive studies that were carried out during World War II The chemical pharmacologic and clinical studies of these compounds have been summarized [8]

One group of alkylating agents the mustards owe their biologic activity to the presence of the *bis* beta-chloroethyl grouping [30] In neutral or alkaline aqueous solution the nitrogen mustards are highly reactive The tertiary amine undergoes intramolecular transformation to form a cyclic onium cation with liberation of a chloride ion This cyclic ethylenimonium cation reacts readily with inorganic anions water and organic radicals [30] These



compounds limit tissue growth by inhibiting mitotic activity and cell division Although the nitrogen mustards are capable of inactivating many enzyme systems the evidence appears to indicate that these compounds do not act in vivo by inhibiting intracellular enzymatic activity [8, 30, 3] Considerable work along different lines has led to the hypothesis that alkylating agents which are active in aqueous solution produce their anti tumor effect by esterifying ionized acid groups rather than by reacting with sulfhydryl groups or free amino groups [3]

A reaction with nucleic acids especially the inactivation of deoxyribonucleic acid would have a profound effect on cell division In vitro experiments have demonstrated that alkylating agents cause marked alterations in the physical properties of nucleic acids *These include changes in the shape* of the macromolecules and in the ability to combine with basic material [53] It has been demonstrated that nitrogen mustard produces a marked decrease in the viscosity of dilute solutions of deoxyribonucleic acid [9] Other in vitro studies that have utilized the electron microscope have demonstrated intra molecular cross linking and coiling up of deoxyribonucleic acid molecules after exposure to nitrogen mustard [57] The alkylating agents appear to act by producing cross linking of the different DNA molecules via their phosphate groups which renders them inactive

The alkylating agents that have been used most widely in clinical practice are

1 Methyl *bis* (β chloroethyl) amine hydrochloride (nitrogen mustard HN)
2 Triethylene melamine (TEM)
3 *p* (Di 2 chloroethylamine) phenylbutyric acid (chlorambucil CB 1348 Leukeran)
4 1-4 Dimethanesulfonyloxybutane (busulfan Myleran)

These compounds differ in their effectiveness against different strains of cells different types of lesions and in their toxic effects

Nitrogen Mustard

Nitrogen mustard has been studied more extensively than the other alkylating agents The histologic changes in the lymphoid tissue and marrow that occur in rats after the injection of mustard were studied by Kindred.[41] He found that lymphocytes were more sensitive than the granulocytes or the

red cell precursors reticulum cells and megakaryocytes were much less sensitive

For clinical use nitrogen mustard is made up in a fresh solution of physiologic saline and is administered intravenously by injecting it into the tubing of an intravenous saline infusion Local thrombophlebitis sometimes develops and a severe local reaction occurs if the nitrogen mustard is extravasated into the tissue Approximately 80 per cent of the patients who receive the drug develop nausea and vomiting [40]

The most important toxic effect is exerted on the bone marrow This is manifested within seven to ten days by a fall in the leukocyte count followed by a decrease in the platelets that generally reaches its lowest level within two or three weeks In order to avoid serious damage to the bone marrow an interval of at least six weeks is allowed between courses of therapy whenever possible When the patient has extensive involvement with lymphosarcoma smaller doses spaced several days apart are advisable because the rapid destruction of tissue may cause hyperuricemia and renal impairment[45] or even vascular collapse [49] Evidence of impaired bone marrow function is also an indication for smaller doses spaced at longer intervals

Nitrogen mustard has found its greatest usefulness in Hodgkin s disease in which it often produces a prompt remission in fever sweats anorexia pruritus and weakness accompanied by a decrease in the size of the spleen liver and lymph nodes In lymphosarcoma reticulum cell sarcoma and giant follicular lymphoma the response to treatment with nitrogen mustard varies greatly in different patients but in general the response is not as good as in Hodgkin s disease [30 38 40 4 59 63 64 6] Nitrogen mustard has not been used widely in the treatment of chronic leukemia though it has been suggested that it might be effective if a satisfactory plan of administration could be worked out [4]

TEM

Triethylene melamine (TEM) is converted in an acid medium to a reactive quaternary ethylenimonium compound and produces therapeutic effects similar to those produced by nitrogen mustard Since it is transformed into its active form in an acid medium it may be administered by mouth Because TEM will be inert by the time it is absorbed if the stomach contents are too acid it is usually administered with sodium bicarbonate It is often difficult to determine how much active compound has been absorbed and in addition there seems to be considerable individual variation in response to the drug For these reasons the drug must be administered with great caution In some patients an initial dose of 2 5 mg produces a marked effect on hematopoiesis that persists for months Although the effects of TEM are similar to those produced by nitrogen mustard, better clinical results have been obtained with TEM in lymphosarcoma and giant follicle lymphoma than in Hodgkin s disease and reticulum cell sarcoma [54] Good clinical results have been obtained in the treatment of both chronic myelocytic leukemia and chronic lymphocytic leukemia with TEM [4]

Chlorambucil

Chlorambucil (CB 1348) a water soluble aromatic nitrogen mustard is also administered orally and is much less toxic to hematopoietic tissue than nitrogen mustard or TEM It can produce marrow aplasia, however and blood counts should be performed at intervals of not less than three weeks while the patient is receiving the drug[37] Improvement generally occurs in three or four weeks if the drug is effective and the usual course of treatment lasts from four to eight weeks Gastrointestinal disturbances are mild and infrequent This drug is more effective in diseases characterized by abnormal lymphocyte proliferation such as lymphosarcoma and giant follicle lymphoma than in reticulum cell sarcoma and Hodgkin s disease[37] However remissions were obtained in 22 of 47 patients with Hodgkin s disease and in 12 the remission lasted five to twelve months[1] Chlorambucil is effective in the treatment of chronic lymphocytic leukemia but is ineffective in chronic myelocytic leukemia

Myleran

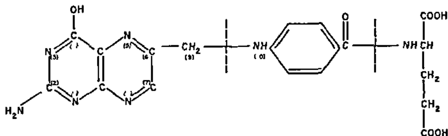
Myleran is a sulfonic acid ester and in low doses selectively depresses granulocytopoiesis This alkylating agent is an effective agent in the treatment of chronic myelogenous leukemia and other myeloproliferative disorders[14, 7] but has not been effective in other forms of malignant disease The ease of administration and the relatively high therapeutic index of Myleran make it the agent of choice in chronic myelogenous leukemia

ANTIMETABOLITES

Folic Acid Antagonists

Folic acid antagonists were introduced in the treatment of acute leukemia by Farber who in 1947 noted the acceleration of the leukemic process in patients receiving folic acid He suggested that antifolic compounds might be of benefit, and the following year reported the production of a temporary remission in 10 of 16 children with leukemia who were treated with an antifolic compound aminopterin an observation which ushered in the present era of chemotherapy in leukemia[19] The use of these agents which interfere with the metabolism of neoplastic cells to a greater extent than they do with the metabolism of normal cells has not yet resulted in the cure of leukemia but it has produced striking temporary remissions in the disease[3, 39, 94]

The antifolic compounds are derived by substitutions in the folic acid molecule The hydrogen atoms in the structure may be replaced as may the hydroxyl on the pyrimidine ring or the glutamic acid may be replaced in its entirety by another amino acid or polypeptide chain Aminopterin is produced by substitution of an amino group for the hydroxyl group A methopterin is formed when in addition to the amino group substitution a methyl group is substituted at position 10



Folic acid

The mechanism by which the folic acid antagonists exert their effect is not understood completely Folic acid is essential for the synthesis of nucleic acid as a result of its effect on formate metabolism The antifolic acid compounds may inhibit the conversion of folic acid to citrovorum factor (folinic acid) and thus limit formate metabolism formate along with CO NH_3 and glycine is essential for the synthesis of deoxyribonucleic acid and ribonucleic acid The antifolic compounds may also interfere with the syn thesis of thymine [8] which is used in the synthesis of nucleic acid It is probable that folic acid antagonists are effective because they disrupt the metabolic pathways that lead to the formation of nucleic acid [6] These drugs exert their greatest effect on cells which are dividing rapidly and have the greatest demand for nucleic acid Ordinarily the cells of the bone marrow lymph follicles and gastrointestinal tract are the first that are affected Leukemic cells which have an extremely high proliferative rate are affected to a greater extent than normal cells It is only the difference in the rate of proliferation that makes leukemic cells more sensitive to these drugs than normal cells Since normal cells are also affected there is a definite limit to the amount of the drug that can be administered

A number of investigators have reported their results with the antifolic compounds [10, 19, 0, 34] Of a group of 425 children with acute leukemia 68 per cent received some benefit and 30 to 50 per cent obtained good clinical and hematologic results Since these preparations are beneficial in only about 14 per cent of the adults [10] their chief use is in the treatment of leukemia in children A methopterin is usually preferred to aminopterin because there is a wider range of safety between the effective therapeutic dose and the dose that produces severe toxic effects [18] Both compounds are administered by the oral route

Purine and Pyrimidine Analogs

Because investigations of nucleic acid synthesis revealed a close relation ship between folic acid purine and thymine metabolism efforts have been made to find analogs of purine and pyrimidine compounds which would block nucleic acid synthesis particularly in malignant cells [6, 35] For years it was thought that dietary purines and pyrimidines could not enter into the formation of nucleic acids [1] After the demonstration of the incorporation of adenine into nucleic acid in 1948 this concept was modified [7] This observa

tion stimulated the search for purine analogs which might effectively interfere with cellular metabolism Each of the naturally occurring purine and pyrimidine bases has been investigated systematically and new compounds have been produced by altering the basic chemical composition of the naturally occurring compounds [3] These compounds have been tested in microbiologic systems and those that showed promise have been tested against tumors or leukemia in rodents These purine antagonists are believed to interfere with nucleic acid metabolism perhaps by becoming incorporated into nucleic acid and rendering it biologically ineffective [6] It has been suggested that a hypoxanthine containing compound that is an intermediate in the conversion of adenine to guanine may be the site of action [17]

The first clinical trials with 6-mercaptopurine were encouraging In 1953 it was reported that 15 of 45 children with acute leukemia underwent a good clinical and hematologic remission and another 10 had a partial remission Resistance to therapy appeared to develop more rapidly than with the antifolic compounds but it was observed that patients who had become resistant to the antifolic acid compounds were still sensitive to treatment with 6-mercaptopurine [3, 9] A review of the results obtained with 6-mercaptopurine indicates that remission will occur in approximately one-third of the children with acute leukemia and in about one seventh of the adults Remission may occasionally be obtained in children who have become resistant to steroid and antifolic acid compounds [3] but a response to 6 mercaptopurine therapy is not usual in children who fail to respond initially to steroids or antifolic acid therapy [11] ° After relapse occurs the subsequent remissions that are obtained with any of these drugs are less complete and of shorter duration than the initial response

8 Azaguanine

8-Azaguanine a compound similar to guanine in chemical structure has been reported to be effective in acute leukemia -3 The compound is interesting because it has been shown to inhibit adenosine deaminase in vitro [4] This observation suggests that compounds may interfere with nucleic acid metabolism by inhibiting essential enzymes It appears that leukemic cells are much more sensitive to 8 azaguanine than are normal cells Toxic manifestations such as dermatitis nausea vomiting and diarrhea have limited the amount of the drug that can be administered

ADRENOCORTICAL STEROIDS

The initial report of the use of adrenocortical steroid compounds in the treatment of a child with acute leukemia was encouraging Administration of the drug was followed by a remission that lasted two months [1] In one series of cases hematologic improvement was noted in 44 per cent of the patients with acute leukemia treated with adrenocorticotropic hormone and 52 per cent of those treated with cortisone From 60 to 70 per cent of the patients

obtained some improvement from both drugs but adults did not respond as well as children Following relapse subsequent remissions became more difficult to achieve Larger series have not revealed such good results In a series of 425 patients only 30 per cent had a good remission and another 24 per cent obtained a partial remission The remission rate was higher in patients with acute lymphoblastic or undifferentiated leukemia Remissions were not observed in myeloblastic or monocytic leukemia and in some patients the disease seemed to be made worse Others[18] report that occasional remissions that do not differ from those seen in other forms are seen in the myeloblastic and monocytic leukemia These preparations may be beneficial in the treatment of an associated bleeding tendency or hemolytic anemia even when they produce no striking change in the leukocyte count

While adrenocortical steroids may occasionally produce a remission in diseases of the lymphoma group they are not as effective as ionizing radiation or the alkylating agents They are particularly useful in the treatment of the auto immune hemolytic anemias which may accompany these disorders

Complications of steroid hormone therapy were more common when only cortisone and adrenocorticotropic hormone were available Electrolyte disturbances with excessive potassium loss and sodium retention were a constant problem Dietary sodium restriction and the administration of oral potassium chloride minimized these dangers but did not remove them With the advent of the newer adrenal steroid analogs the problem of electrolyte imbalance has ceased to be of clinical importance However other dangers common to all of the adrenocortical hormones remain These compounds may so alter the usual body responses to infection or to peritoneal soiling that the occurrence is masked An increased incidence of peptic ulceration has been observed and in some patients severe gastrointestinal hemorrhage occurs The development of serious systemic or local fungus infection is not unusual in patients receiving these compounds Changes in mood are seen commonly and major psychotic reactions may occur The administration of adrenocortical steroids will curtail endogenous steroid production and for this reason it is advisable to reduce the drugs slowly if therapy is terminated Weeks or months after cessation of therapy with these preparations the severe stress of a surgical procedure or infection may precipitate an Addisonian crisis despite the adequacy of adrenal function for ordinary day to day activities

The possible mechanisms by which these compounds might produce an effect on the hematopoietic system have received wide attention[33] Patients with adrenal insufficiency are known to have a lymphocytosis while those with adrenal hyperplasia have a lymphopenia The administration of adrenocorticotropic hormone or adrenocortical steroids will depress the lymphocyte count Stress reactions in the intact animal produce a lymphopenia whereas the adrenalectomized animal responds to the same stimulus with a lymphocytosis[16] Evidence has been presented which suggests that the adrenal steroids exert a destructive effect on lymphoid tissue[16] and a cyto

lytic effect on the individual lymphocyte[26] or influence the distribution of the lymphocytes in the body[33] It is possible that all of these effects contribute to the lymphopenic state that is produced by these compounds The effect of the adrenal gland on myeloid activity is less clear While there have been reports that increased myeloid activity occurs as the result of the administration of adrenocortical steroids to humans[48] a decrease in myeloid activity during prolonged administration of these compounds to laboratory animals has also been observed[3 33] The mechanism through which the adrenocortical hormones favorably influence the bleeding tendency and hemolytic anemia which may occur in patients with leukemia and malignant lymphoma is likewise unknown

During the administration of these compounds a distinct elevation in the mood of the patient often occurs Appetite improves and a sense of well being is noted Fever tachycardia and toxicity" diminish These improvements often allow time for one of the cytotoxic agents to exert an effect

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CHAPTER X

Leukemia

Historical Aspects

IN 1845 Bennett[4] Craigie[16] and Virchow[101] independently reported patients with undoubted leukemia Bennett and Craigie attributed the remarkable numbers of white blood cells to the presence of "purulent matter in the blood." In 1846 Virchow who introduced the term "leukemia" did not agree that the changes in the blood were the result of the invasion of the blood stream by a suppurative process and considered that the hematologic changes were part of a definite pathologic process that involved certain organs in the body The conflicting claims regarding the priority of discovery of leukemia were reviewed by Osler[66] who concluded that Virchow deserves priority because he was the first to realize that the increase in white blood cells was an essential feature of the disease to which he gave the name leukemia" Two forms of leukemia were recognized by Virchow In one the small forms of white cells predominated and enlargement of the lymph nodes was com mon in the second the large white cells were increased in number and marked splenomegaly occurred Acute leukemia was first described by Friedrich in 1857[19] [9] and in 1913 the first case of monocytic leukemia was reported by Reschad and Schilling Torgau

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Although our knowledge of many essentials of the leukemic process remains inadequate, a considerable body of information has accumulated relative to the production of leukemia by external agents and the genetic predisposition to leukemia. Much of this evidence has been derived from the study of laboratory animals, but important deductions have also been drawn from consideration of the occurrence of the disease in man.

1 *Ionizing Radiation*

Evidence from a number of sources indicates that ionizing radiation can be a leukemogenic agent in man as well as in mice. The incidence of leukemia in physicians is twice that of the general population, and the incidence in radiologists is from eight to nine times that in physicians in general.[59] An increased incidence of leukemia in survivors of atomic bombings in a frequency directly related to the severity of the exposure to radiation appears to be well established.[27, 48, 8] The therapeutic use of x ray for spondylitis in adults[12] and for thymic enlargement in children[9] has been reported to be responsible for leukemia. It is suspected that the incidence of leukemia, particularly acute leukemia, is higher in patients with polycythemia vera who have been treated with x ray or radioactive phosphorus than in those treated by other means.[84] Even the use of diagnostic x ray during pregnancy has been suspected as a factor in the development of leukemia in childhood.[43, 93]

2 *Drugs*

The evidence that exposure to benzol (benzene) may produce leukemia is circumstantial, but a number of suspicious instances have been reported.[46] The individual response to this chemical appears to vary and both the concentration and length of exposure are probably important.

3 *Infections*

Severe infections of various types usher in some 50 per cent of the cases of acute leukemia in childhood.[4] Some authors have suspected infection may cause leukemia, but most workers have concluded that such infections occur as a consequence of the preexistent leukemia.[81] While the chance that bacterial infection may be of etiologic significance seems remote, the possibility that a virus may cause leukemia has received much support.

Studies of the role of viruses in leukemia have been carried out largely in mice and fowl, and the relationship of the leukemia that occurs in these species to that of man is still uncertain.[8] Gross[38] has shown that leukemia may be transmitted to mice by the injection of a suspension of cells or cell-free extracts. He has suggested that a causative virus might be transmitted from parent to offspring and remain dormant for some time. Cell-free extracts of liver, spleen, or lymphatic tumors of AKR* mice have produced leukemia

* AKR strain—mice having a high incidence of spontaneous leukemia.

The Incidence of Leukemia

It is reported that in the last 50 years there has been a threefold to sixfold increase in leukemia the greatest increase that has occurred in any disease except coronary thrombosis and carcinoma[47] In England and Wales the incidence of leukemia has increased from 1 7/100 000 in 1900 to 5/100 000 in 1955[68] while in the United States comparable studies indicate an increase from 1/100 000 in 1900 to 6 3/100 000 in 1952. Despite such figures as these and the distinct impression of many workers interested in the subject that leukemia is increasing in frequency Dameshek and Gunz conclude that as yet an actual as opposed to an apparent rise has not been proved.[0] Others believe that such factors as improved diagnosis more frequent hospitalization improved treatment and the increased number of older persons in the general population do not explain the apparent increase[1, 4] In the period 1943 1952 the incidence of leukemia in males was 7 3/100 000 while that for females was 5 77/100 000[36, 46, 50] The disease is more common in Jews than in non Jews and is less common in Negroes than in Caucasians[7] The incidence in Caucasians and Negroes is about the same if the Jewish population is excluded

Approximately 60 per cent of patients with leukemia have the chronic form of the disease which occurs more commonly after middle age Chronic myelocytic leukemia is slightly more common than chronic lymphocytic leukemia Acute leukemia occurs most commonly in children and young adults but an increasing incidence in older persons has been reported[1, 4]

ETIOLOGY AND PATHOGENESIS

In 1894 Osler wrote of leukemia "Notwithstanding most careful clinical study and thorough histological and bacteriological investigation the secret of the causative factor of this disease is as profound as it was half a century ago"[66] Unfortunately the "secret of the causative factor" of leukemia is still a mystery

Although the cause of leukemia is unknown there is considerable evidence to support the view that leukemia is a neoplasm[0, 31, 4] The essential alteration is thought to reside in the leukemic cell and be responsible for the failure of the cell to respond to the forces which ordinarily control its reproduction and maturation The autonomous uncontrolled behavior of these cells appears to be responsible for the clinical manifestations and course of the disease Whether a single factor or multiple factors are responsible for the essential alteration of the cell is unknown Because of ignorance on this point the question arises whether or not all of the clinically recognized forms of acute and chronic leukemia are the same disease Different types of leukemia differ considerably in age distribution clinical manifestations survival time and response to various types of therapy However these differences could be explained by a varied host response as well as by multiple causative factors

The production of leukemia by the injection of cell free chromatin particles and microsomal fragments into mice has been reported.[97] It has been suggested that the chromatin particles enter normal cells and alter their function in some undefined manner. The significance of the reported transfer of the neoplastic character of a cell in this manner cannot be determined until further studies that utilize genetic markers are carried out.[9]

The increased cell counts seen in leukemia have been interpreted as a failure of normal elimination of the leukocytes rather than an overproduction of these cells.[9] It has been shown that the lungs, liver, and gastrointestinal tract daily remove large numbers of cells from the circulation. Cross circulation experiments have demonstrated that the rate of removal of cells from the circulation is decreased in patients with leukemia as compared to the normal.[9] The relationship of this defect to the pathogenesis of leukemia is not established.

In the concept that leukemia is a deficiency disease the immaturity of the white blood cells is considered to be the result of a maturation arrest similar to that seen in the red cell precursors in pernicious anemia. Thus far all attempts to establish leukemia as a deficiency disease have failed.[9] While a number of metabolic differences between normal and leukemic cells have been demonstrated, none has yet been established to be a cause rather than an effect of leukemia.

There is yet insufficient evidence to support more than speculation as to the nature and cause of human leukemia. However, the evidence that a virus may play a causative role cannot be ignored. It seems reasonable that a virus-like agent might significantly alter the metabolic activities and behavior of a host cell. Genetically determined host resistance must be of great importance and might well be influenced by ionizing radiation and chemical agents, which would account for the demonstrated role of these factors in leukemia.

ACUTE LEUKEMIA

All types of acute leukemia, myeloblastic, lymphoblastic and monoblastic, are much more severe diseases than any of the chronic leukemias. The illness may begin as acute leukemia or the acute form may develop as the terminal event in a patient who has had the chronic form of the disease for months or years. The presence of acute leukemia is characterized by the occurrence of appreciable numbers of young cells, particularly the blast forms, in the circulating blood and bone marrow. The different types of acute leukemia cannot be distinguished from one another with certainty on clinical grounds alone, and because of the extreme immaturity of the cells it may be difficult to identify the cell type even after careful hematologic study. The lymphoblastic type is more common in children, the myeloblastic is fairly evenly divided among the different age groups, and the monocytic type occurs most often in middle age.[illegible] All types of acute leukemia are more common in males.

and epitheliomas when injected into newborn mice of the C3H† strain less than 16 hours old[39] This observation suggests a relationship between leukemia and other malignancy in mice a relationship that is well recognized in man[64, 100] Other reports have indicated that injections of cell free extracts of brain from mice or human beings with leukemia cause the development of leukemia in a strain of mice not ordinarily susceptible to the disease[6, 68] These and other studies demonstrate that leukemia can be produced in both mammals and fowl by the injection of cell free filtrates Should a virus be shown definitely to be the agent responsible for leukemia the relationship of the virus to continued neoplastic activity of the cell would remain to be clarified[19]

4 *Genetic Considerations*

In certain highly inbred strains of mice leukemia develops spontaneously in from 60 to 70 per cent of the animals This suggests that genetic factors are important under conditions of controlled breeding and that man may be protected by his varied ancestry Some constitutional susceptibility to leukemia in man was suggested by the studies of Videbeck[100] but later studies have not confirmed his conclusions[40] Reports of the occurrence of several instances of leukemia in the same family suggest a genetic influence but are not conclusive because some unrecognized factor in the family environment may be responsible for the disease[1] Observations on the occurrence of leukemia in identical twins do not afford definite evidence of the existence of a hereditary factory in leukemia[19] In the rare instances of congenital leukemia that have been reported the disease has not been demonstrated in the mother[74] There has been a recent report of the occurrence of leukemia in a nine month old child born of a leukemic mother[17] In all other reported instances of children born of leukemic mothers the offspring have remained free of the disease[8]

5 *Other Factors*

It has been suggested that leukemia is the result of an imbalance in the hematopoietic equilibrium This concept was suggested by the identification of two chemicals myelokentric acid and lymphokentric acid in the urine of patients with leukemia or lymphoma[61] Recently the same materials have been obtained from the sera of patients with these diseases[30] It is reported that myelokentric acid produces hyperplasia of myeloid elements and lymphokentric acid produces hyperplasia of the lymphoid elements when injected into the guinea pig[61] It has been suggested that an imbalance in the production of these "humoral hormones causes leukemia but the evidence is not convincing

† C3H strain—mice having a low incidence of spontaneous leukemia

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Clinical Manifestations

The clinical characteristics of acute leukemia vary from case to case and depend mainly on the organ systems involved in the disease. The clinical manifestations in patients who have acute leukemia from the onset differ from those that are seen in patients who undergo a transformation from the chronic to the acute form of the disease.

In most children and young adults the disease is acute from the onset, but the initial symptoms may give no hint of the seriousness of the underlying disease. In children especially the onset may be characterized by the development of listlessness, fretfulness, poor appetite and pallor. In other instances aching in the extremities or pain from a pathologic fracture, particularly of a vertebra, may be the initial manifestation. The onset of the disease often resembles that of an acute respiratory infection with fever, malaise, fatigability and sore throat, or may mimic some severe infection with high fever, headache, sweats and severe prostration. Such episodes often occur as a result of infections that develop because of the lowered resistance of these patients, but also occur in the absence of demonstrable infection.[18, 9, 93] Abnormal bleeding, such as epistaxis, bleeding gums, ecchymoses or petechiae, occurs some time in the course of the illness in nearly all patients *with acute leukemia and may be present from the onset.*

The transformation of a chronic leukemia into the acute variety is seen most often in adults with chronic myelocytic leukemia. The change is usually accompanied by increasing weight loss, fever, progressive anemia and the appearance of a hemorrhagic tendency. A change in type is recognized by a change in the morphology of the peripheral blood and bone marrow that consists of increased numbers of the more immature cells, myeloblasts or promyelocytes. The hematologic changes may be noted in some patients before the changes in the clinical course are apparent.

Many of the clinical manifestations are the result of the involvement of specific organs by the leukemic process. Hemorrhage or infiltrations in the brain and *other parts of the central nervous system occur frequently.* The incidence of objective signs of neurologic involvement (other than retinal changes) was reported to be 20 per cent in one series of 334 cases.[53] Convulsions, sensory disturbances, cranial nerve palsies and signs of pyramidal tract involvement are not uncommon. Infiltration in other organs such as the bladder and kidney may produce pain and hematuria. Severe pain may develop about the joints and in the extremities as *a result of involvement* of the synovial membranes or as a result of subperiosteal infiltration or hemorrhage. Bleeding from the gastrointestinal tract occurs frequently and may be severe.

Physical examination generally reveals marked pallor of the skin and mucous membranes. Petechial hemorrhages and ecchymoses of different sizes occur commonly in all parts of the skin and mucous membranes. Leukemic infiltration of the skin, which has a characteristic purplish color, occurs occasionally but is less common than in chronic leukemia. Not infrequently the gums appear hypertrophic, swollen and ulcerated, a finding that occurs

more often in monocytic leukemia than in the other types[11] The gums bleed easily when touched and continued spontaneous oozing develops in many patients Retinal hemorrhages are often present.

A soft spleen edge is usually but not invariably palpable a few centimeters below the left costal margin Splenomegaly occurs less often in adults than in children with acute leukemia[4] In adults the observation that the spleen edge is very firm and extends as far down as the umbilicus suggests that the patient has had a chronic leukemia before the onset of the acute phase In acute leukemia enlargement of the liver is inconstant and hepatic tenderness is unusual[19, 6] Mild or moderate (0.5 to 1.5 cm) enlargement of the superficial lymph nodes is usually present but the enlargement may be so slight as to escape detection Tenderness on light pressure over the bones particularly the lower portion of the sternum is commonly demonstrable in acute leukemia

Chloromas tumors that consist of masses of myeloblasts occur most often in children and young adults They usually involve the bones of the face and cranium particularly the orbit[9] The tumors have a characteristic green color that fades rapidly on exposure to air Hyposulfite and hydrogen peroxide restore the green color[9, 9]

Laboratory Manifestations

Anemia which may result from blood loss decreased blood production increased hemolysis or a combination of these factors occurs in almost every patient with acute leukemia The anemia is often severe and hemoglobin levels as low as 2 to 3 grams per 100 ml are sometimes seen The erythrocyte count and hematocrit are reduced proportionally and the anemia is generally of the normocytic normochromic variety

The most important abnormality in acute leukemia involves the leukocytes The total leukocyte count varies from subnormal levels of a few hundred to several hundred thousand per cu mm Characteristically very immature cells occur in significant numbers Often in smears prepared with Wright's stain the identity of the young cells can only be surmised by a



Figure 53 Blast cells with Auer bodies A, Monoblasts from patient with acute monocytic leukemia B Myeloblasts from patient with acute myeloblastic leukemia

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Clinical Course

Acute leukemia is a uniformly fatal disease that usually runs a short course Before the widespread use of transfusions and the introduction of antibiotics and chemotherapeutic agents the survival time was usually from six days to six weeks Although there is some difference of opinion as to the effectiveness of the newer methods of therapy in prolonging the survival time of the patient with the disease the course seems to be prolonged in some individuals for a matter of months and sometimes for years[44, 99] Practically complete clinical and hematologic remissions may be produced by the methods now used in treatment, but unfortunately the ultimate outcome remains the same and death usually occurs as the result of hemorrhage or infection

Treatment

The treatment of acute leukemia involves the use of both supportive measures and specific therapy Included in the former are the use of blood transfusions for the anemia fresh blood collected in plastic bags for thrombocytopenia and bleeding antibiotics for associated infection and analgesics for pain Among the most important supportive measures is the attitude of the physician toward the patient and his family The human interest stories in the press have made leukemia a tragic story known to nearly all The physician's support of members of the family his sympathetic understanding of their anxieties and his willingness to take the time to talk with them contribute greatly to their comfort It is important that the parents of children with leukemia be urged to treat them during periods of remission as they would a normal child The children should be allowed to return to school and play and normal discipline should be enforced In this way the over all emotional impact of the disease on the family may be reduced

Among the specific measures used in treatment are (1) folic acid antagonists (2) purine antagonists and (3) adrenocortical steroids Adults do not respond nearly as well to these "specific measures as children do Even in children remissions are generally of short duration and it is necessary to change from one preparation to another or to administer several agents concurrently as the disease becomes refractory to the drug that is being used Although all of the remissions to date have been temporary and relatively short the fact that they have occurred has encouraged the hope that more prolonged and lasting remissions will become possible with newer agents

A Methopterin The anti folic acid agent of choice is a meth[illegible] (Methotrexate) which seems to have a higher therapeutic [illegible] aminopterin The average daily oral dose of a methopterin for a [illegible] old child is 2.5 to 5 mg The recommended initial dose for adults [illegible] per day and if neither toxicity nor improvement occurs in four to [illegible] it is increased gradually The drug is administered continu[illegible] desired clinical effect is obtained or until evidence of tox[illegible]

positive identification of the older cells that are seen The use of the peroxidase stain is often helpful since the finding of peroxidase positive granules in the young cells indicates a myelogenous or monocytic origin of the cell The presence of Auer bodies also places the young cells in the myelocytic or monocytic series On occasions only very immature atypical blast cells are found and the type of leukemia cannot be identified with assurance such cases are often classified as "stem cell" leukemia When the total leukocyte count is low the examination of smears made from a concentrate of the leukocytic fraction after centrifugation of the peripheral blood is often useful in demonstrating the presence of immature cells Study of the peripheral blood in this manner often makes it possible to distinguish between leukemia and aplastic anemia and is also helpful in identifying the type of leukemia Numerous nucleated red cells are seen commonly in the peripheral blood in all types of acute leukemia

Thrombocytopenia occurs sooner or later in nearly all types of acute leukemia Usually the platelets are markedly reduced or absent when the patient is first seen

Examination of the bone marrow generally reveals infiltration by the immature leukemic cells that are responsible for the disease In all types of leukemia there is marked involvement of the bone marrow which may be filled with the abnormal cells at a time when there is but little or equivocal evidence of the disease in the peripheral blood

Diagnosis

The diagnosis of acute leukemia is generally made without difficulty when there is a marked increase in the immature leukocytes in the peripheral blood and bone marrow associated with anemia and thrombocytopenia. On occasion acute leukemia must be distinguished from infectious mononucleosis The absence of anemia and thrombocytopenia the absence of true blast cells in the peripheral blood the varied morphologic appearance of the cells in the peripheral blood the failure to find evidence of significant marrow involvement and the positive reaction with the heterophil agglutination test usually serve to distinguish infectious mononucleosis without much difficulty (see Fig 51) Aplastic anemia offers more difficulty because anemia, leukopenia and thrombocytopenia fever and abnormal bleeding may occur in both diseases The presence of lymphadenopathy splenomegaly the appearance of the bone marrow and the study of the smear of the peripheral blood made from a concentrate of the leukocytes nearly always enable one to make the correct diagnosis but occasionally a period of observation is necessary before this can be done Leukemoid reactions in infections usually cause no difficulty because the proportion of very immature cells in leukemia is generally out of all proportion to that seen in severe infections where there is a more orderly increase in the intermediate and immature forms

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The treatment of acute leukemia involves the use of both supportive measures and specific therapy. Included in the former are the use of blood transfusions for the anemia, fresh blood collected in plastic bags for thrombocytopenia and bleeding, antibiotics for associated infection, and analgesics for pain. Among the most important supportive measures is the attitude of the physician toward the patient and his family. The "human interest" stories in the press have made leukemia a tragic story known to nearly all. The physician's support of members of the family, his sympathetic understanding of their anxieties, and his willingness to take the time to talk with them contribute greatly to their comfort. It is important that the parents of children with leukemia be urged to treat them during periods of remission as they would a normal child. The children should be allowed to return to school and play, and normal discipline should be enforced. In this way the over-all emotional impact of the disease on the family may be reduced.

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A-Methopterin. The anti-folic acid agent of choice is a-methopterin (Methotrexate), which seems to have a higher therapeutic index than aminopterin. The average daily oral dose of a-methopterin for a five-year-old child is 2.5 to 5 mg. The recommended initial dose for adults is 5 mg per day, and if neither toxicity nor improvement occurs in four to five weeks it is increased gradually. The drug is administered continuously until the desired clinical effect is obtained or until evidence of toxicity forces dis-

continuance of the medication If a remission is produced it is recommended that a maintenance dose be given until the patient becomes refractory or some complication develops[23] If signs of toxicity force discontinuance of the drug it is restarted at a lower dose when the signs have cleared It has been emphasized that a patient may have several episodes of toxicity before a remission occurs

The toxic effects of these drugs include ulceration of the mouth anorexia ulceration and hemorrhage of the gastrointestinal tract nausea vomiting leukopenia thrombocytopenia and pancytopenia There may be loss of hair and rarely disturbance of liver function The toxic effects seem to be more prominent in adults than in children

6 Mercaptopurine The most commonly used purine antagonist is 6 mercaptopurine The recommended oral dose is 2 5 mg per kilo per day and is calculated to the nearest 25 mg It is administered as a single dose at any time of the day The average five year old child receives 50 mg per day while the average adult receives 100 to 200 mg per day The time required for an initial effect of the drug is often between two and four weeks but at times a rapid fall in the number of leukocytes in the peripheral blood occurs within a few days and the leukocyte count should be followed carefully If response has not occurred after the drug has been used for four weeks the dose should be increased to 3 to 5 mg per kilo per day[23] If a remission is obtained a maintenance dose is continued

6 Mercaptopurine is usually well tolerated and serious toxic reactions are unusual The most important of these is depression of the bone marrow However the development of leukopenia does not always indicate that bone marrow depression has occurred since the leukopenia may be simply an early response to the drug When bone marrow depression occurs it usually responds promptly to withdrawal of the drug[6] Megaloblasts are seen in marrow during therapy with anti folic acid compounds but are not seen in the marrows of patients treated with 6 mercaptopurine Anorexia mild nausea and occasional vomiting sometimes develop during the use of 6-mercaptopurine but oral lesions and severe gastrointestinal upsets are not produced

Adrenocortical Steroids Adrenocorticotropic hormone (ACTH) or adrenocortical hormones have been more effective in the treatment of acute leukemia in childhood than in adults but remissions have been obtained in both They are particularly useful when on admission the patient adult or child is extremely ill or has a bleeding tendency

If there is great urgency 25 to 50 mg of ACTH may be given over a 24 hour period by continuous intravenous drip Hydrocortisone may also be given intravenously in doses of 100 to 200 mg daily

Among the oral preparations available prednisone has proved useful and is unlikely to produce electrolyte disturbances The initial oral dose of prednisone is 60 to 80 mg per day The drug should be given in divided doses at eight hour intervals The dosage is reduced as improvement occurs and 15 to 20 mg per day is often sufficient for maintenance therapy

Illustrative Case

This 44 year old surgeon reported that he was in his usual health until about two weeks prior to his visit when he first noted malaise and easy fatigue Two or three days before his examination he noticed petechial lesions on the hands while he was scrubbing for an operation and this led him to seek medical advice

Physical examination revealed no significant abnormality The patient did not appear pale had no significant lymphadenopathy retinal hemor rhage or hepatomegaly Roentgenogram of the chest was normal

Laboratory Examinations Hematocrit was 36 per cent erythrocyte count 3 7 mil. per cu mm hemoglobin 12 1 gm per 100 ml leukocyte count 67 500 per cu mm Study of the peripheral blood revealed that all of the leukocytes were blast cells most of them gave a positive peroxidase re action Some cells were binucleated and others contained unusually large nucleoli. Examination of aspirated bone marrow revealed that the marrow was packed with early progranulocytes and myeloblasts The platelets were markedly reduced bleeding time was 8 minutes (normal 1 to 3 minutes) and the prothrombin time was 34 seconds (control of 15 seconds)

Course The patient was made acquainted with his diagnosis of acute myeloblastic leukemia. He decided to postpone any treatment until he had put his affairs in order and returned home the same day On the following day he drew up his will put his financial affairs in order and arranged for the care of his patients Twenty four hours afterward he became partially blind because of extensive bilateral retinal hemorrhages He refused hos pitalization and within a few hours massive gastrointestinal and intracranial bleeding occurred On the following day four days after the diagnosis was made and about three weeks after the onset of his first symptoms the patient died

Comment. The case illustrates the short fulminating course which acute leukemia sometimes takes with or without therapy The hematologic ab normalities—anemia thrombocytopenia and marked increase in leukocytes that consist nearly entirely of blast cells—are typical Death in such patients is usually due to hemorrhage or septicemia

CHRONIC LYMPHOCYTIC LEUKEMIA

In 1924 Minot and Isaacs[63] presented data on 98 patients with chronic lymphocytic leukemia They noted that the disorder occurred most commonly between 45 and 55 years of age a decade later than the peak incidence of chronic myelogenous leukemia. The disease occurred in males more than three times as often as in women In 1953 Shimkin Lucia Appermann and Metter[90] reviewed a series of 137 patients with chronic lymphocytic leukemia and found that the peak incidence was 60 years Males predominated in a ratio of 2 5 1 Scott[89] in 1957 reviewed a series of 227 patients and found the greatest number of cases occurring between the ages of 60 and 70 years He noted that there seemed to be a growing number of women

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leukemia either at the onset or later in the course of the disease.[33] Non specific lesions such as pruritus areas of hyperpigmentation macular and papular eruptions exfoliative dermatitis and herpes zoster occur without actual leukemic infiltration of the skin.[35] Specific lesions the result of actual infiltration of the skin by leukemic cells are usually discrete and bright red or purple in color. These lesions which vary greatly in number usually measure a few millimeters in diameter. Generalized leukemic involvement of the skin—leukemia cutis universalis—generally produces intense itching.

Rarer manifestations of chronic lymphocytic leukemia are massive gastro intestinal hemorrhage,[68] visual disturbances and complaints referable to the skeletal system. Skeletal lesions such as rarefaction of the bones pathologic fracture and collapse of vertebrae with cord compression occur in 5 per cent of the patients with this disease.[59]

On physical examination the most constant abnormality is enlargement of the superficial lymph nodes. In practically every patient some enlarge ment of at least a few of the superficial lymph nodes occurs. In most patients the individual nodes are moderately enlarged and measure from 2 to 3 cm in diameter. The nodes are moderately firm freely movable and non tender. Clusters of nodes may measure 10 cm or more in diameter. Splenomegaly occurs in nearly all patients with chronic lymphocytic leukemia. Usually the spleen is moderately firm and extends several centimeters below the left costal margin but occasionally it fills the entire left side of the abdomen. Hepatomegaly is also an almost constant finding. Retinal hemorrhages may

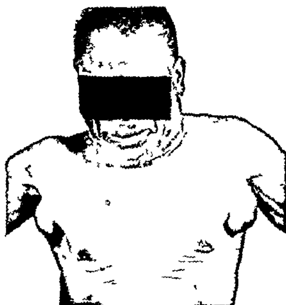


Figure 54 Greatly enlarged axillary and cervical lymph nodes in a patient with chronic lymphocytic leukemia

with chronic lymphatic leukemia In 1929[69] a small series revealed a male female ratio of 4 1 in the two series quoted the ratios are 3 1 and 2 5 1 in Scott s more recent series the ratio was more nearly 2 1

Clinical Manifestations

Chronic lymphocytic leukemia may make its appearance in a variety of ways The onset is sometimes so insidious that the diagnosis is made before symptoms appear when an individual undergoes a routine examination or consults his physician for some unrelated complaint In such circumstances the usual manifestations are previously unrecognized enlargement of the superficial lymph nodes and a moderate increase in the leukocyte count accompanied by an absolute increase in the small lymphocytes Probably the most common complaint that leads the patient to seek medical advice is the accidental discovery of enlarged superficial nodes Any group of nodes may be involved but enlargement of the cervical nodes is most often noted At times the initial complaint is that of involvement of the lacrimal and salivary glands—Mikulicz s syndrome[4] Enlargement of the intra abdominal lymph nodes produces gastrointestinal or abdominal complaints while involvement of the mediastinal nodes may result in cough hoarseness or dyspnea

Abnormalities in the peripheral blood are responsible for the initial symptoms in some patients If anemia is severe easy fatigue exertional dyspnea and weakness occur Thrombocytopenia may cause bleeding gums easy bruising petechiae or gastrointestinal bleeding

A variety of skin lesions have been observed in chronic lymphocytic

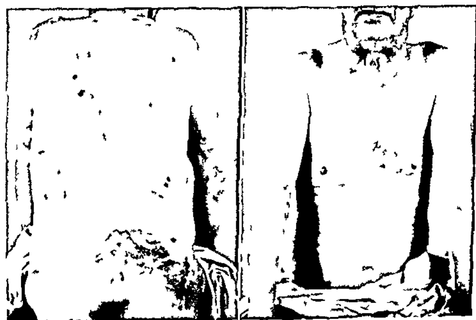


Figure 53 Leukemia cutis and herpes zoster in a patient with chronic lymphocytic leukemia

Infectious mononucleosis is rare in persons over 40 years of age and lymphocytic leukemia is seen most frequently in older people Abnormal appearing lymphocytes of various sizes particularly large cells are characteristic of infectious mononucleosis whereas in chronic lymphocytic leukemia the blood smear contains mainly small lymphocytes or smudge forms The heterophil agglutination test which is almost invariably negative in leukemia is most helpful in younger patients At times the differential diagnosis between lymphosarcoma and the early stage of chronic lymphocytic leukemia is difficult If lymphocytosis in the peripheral blood is inconstant and involvement of the bone marrow is not striking lymphosarcoma should be suspected The diseases cannot always be distinguished even after biopsy of a lymph node

Clinical Course

The average survival time of the patient with lymphocytic leukemia is about 3 5 years[93] but some patients have survived almost 30 years[60] and died from other disorders The three year survival rate is about 50 per cent and the five year survival is about 25 per cent

Many patients with chronic lymphocytic leukemia lead practically normal lives over a course of many years Generally however the disease progresses the lymph nodes liver and spleen increase in size and other manifestations of the disease such as weakness loss of weight anemia, and bleeding appear The appearance of anemia and thrombocytopenia is associated with a relatively poor prognosis Failure of the nodes to regress with therapy the appearance of skin lesions and the occurrence of serious bleeding are also signs of more rapid progression of the disease Death is usually due to cachexia infection or hemorrhage

The occurrence of auto-immune hemolytic anemia is a serious development[5, 7, 58] When the anemia is severe weakness palpitation fatigue and obvious jaundice may be present Fortunately the hemolytic anemia usually responds to adrenocortical steroid therapy Whether or not this type of anemia occurs more frequently in patients treated with radiation therapy is uncertain

Patients with chronic lymphocytic leukemia appear to be more susceptible to bacterial infections particularly pulmonary infection than patients with chronic myelocytic leukemia are[49, 103] The susceptibility to infection in lymphocytic leukemia is thought to be related to the poor response to antigenic stimuli that is manifested by patients with this disease[4, 49]

Therapy

Most reports indicate that treatment of chronic lymphocytic leukemia has not produced any increase in the life span of the patient[63, 90] Osgood however reports that therapy with radioactive phosphorus has resulted in a longer life span than that expected from the natural history of the disease[67] Although there may be some question whether or not any treatment can

occur without other evidence of abnormal bleeding or in association with ecchymoses petechiae and bleeding from the mucous membranes Leukemic infiltration of the tonsil occurs in a small percentage of the patients and can usually be recognized by its reddish purple color The leukemic infiltrations of the skin are usually recognized by the reddish purple color and discrete nature of the lesions If severe pruritus and excoriation are present the underlying nature of the skin lesions may not be apparent

Laboratory Manifestations

The most characteristic features of chronic lymphocytic leukemia are increases in the total leukocyte count and the absolute number of small lymphocytes *Severe leukopenia rarely occurs in chronic lymphocytic leukemia* The leukocyte count is nearly always above 15 000 per cu mm. and often reaches levels of from 200 000 to 400 000 per cu mm The majority of the cells in the peripheral blood are small lymphocytes These constitute 65 to 75 per cent of the cells in the early stage of the disease and usually amount to 95 to 98 per cent of the cells in the more advanced stages A small number of larger lymphocytes occur at times but the presence of blast forms is unusual

In some patients anemia is absent when the patient is first seen but in nearly every patient it develops some time during the course of the disease The anemia may be the result of leukemic infiltration of the bone marrow hemorrhage or hyperhemolysis Unless there has been chronic blood loss the anemia is usually normocytic and normochromic in type Severe anemia with the hemoglobin *as low as 3 or 5 gm per 100 ml is sometimes seen* but more often the hemoglobin is above 7 5 gm per 100 ml If a hemolytic type of anemia is present it is usually associated with reticulocytosis polychromatophilia mild macrocytosis and the presence of nucleated red cells in the peripheral blood The hemolytic anemia in chronic lymphocytic leukemia is most often of the auto immune type and the Coombs test is usually positive Circulating hemagglutinins are demonstrable in some patients [77]

Other abnormalities occur less commonly than the changes in the leukocytes and red cells Among these is an elevation of the basal metabolic rate *to plus 20 to 40 per cent which occurs frequently when the leukemic process* is active A minority of patients with chronic lymphocytic leukemia show disturbances in the plasma protein that resemble those seen in patients with myeloma Although the platelet count is usually normal in patients with chronic lymphocytic leukemia not infrequently thrombocytopenia develops late in the disease [59]

Diagnosis

The diagnosis of chronic lymphocytic leukemia is made without difficulty in most patients because generalized lymphadenopathy leukocytosis and lymphocytosis are usually present At times it may be difficult to distinguish between lymphocytosis due to leukemia and that due to some other cause

body weight per day in a single daily dose This dose is continued from three to six weeks or until a satisfactory response occurs Treatment may then be discontinued until relapse occurs or maintenance therapy may be instituted. In the latter program the dose varies from 2 to 6 mg per day to 3 mg per week. The relative merits of these programs has not been established Chlorambucil is not effective in all patients with chronic lymphocytic leukemia but in our clinic it has appeared to be superior to TEM or nitrogen mustard.

Adrenocortical Steroids In one report adrenocortical steroid therapy with cortisone ACTH prednisone or prednisolone brought about a satisfactory remission in 50 per cent of the patients with chronic lymphocytic leukemia However many of these patients do not respond significantly to these drugs and their greatest use is in the treatment of the complicating hemolytic anemia or thrombocytopenia. Prednisone is started in a dose of 20 mg every eight hours for the first three or four days The dose is then reduced gradually to the lowest dose that controls the hemolytic process or the abnormal bleeding The maintenance dose varies widely In one patient with chronic lymphocytic leukemia and hemolytic anemia under our observation for a long period a single dose of 2.5 mg of prednisone every 24 hours eliminated the necessity of transfusions while complete omission of this preparation was followed by rapid increase in the anemia

As in other forms of leukemia, the attitude of the physician is of great importance in the management of the patient. Other supportive measures include whole blood transfusions to replace blood loss from hemorrhage or infusions of packed red blood cells to correct anemia Antibiotics should be employed as indicated in the treatment of infection Splenectomy is rarely necessary to control hemolytic anemia and thrombocytopenia in patients with chronic lymphocytic leukemia since these complications are usually effectively treated with the adrenocortical steroids

Illustrative Case

This 56 year old male real estate broker was in good health until January 1948 when he consulted his physician because of the sudden onset of left upper quadrant pain which was made worse by inspiration or bending.

Physical examination revealed the patient to be well developed and nourished. Lymph nodes that measured 1 or 2 cm. in size were present in the left supraclavicular region in both posterior cervical regions and in both inguinal areas A firm tender spleen edge extended 6 cm. below the left costal margin The liver was not palpable and the remainder of the physical examination was non-contributory

Laboratory Examination Leukocyte count was 140 000 per cu mm practically all the cells were small lymphocytes hemoglobin 13 gm. per 100 ml erythrocyte count 3.8 mil per cu.mm.

Treatment and Course. After the acute symptoms of splenic infarction subsided, the patient was treated by conventional roentgen ray therapy with 400 r to the splenic area. He improved promptly and two months later the

prolong the course of this disease in all patients it certainly appears to have that effect in some For most patients treatment shortens the periods of incapacity and prolongs the "effective" life span

In patients with chronic lymphocytic leukemia who are asymptomatic and who have only mild enlargement of the superficial lymph nodes accompanied by a mild leukocytosis and lymphocytosis it seems advisable to defer specific treatment until progression of the disease occurs Patients in this category may survive from 10 to 20 years and lead an entirely normal existence without therapy More often the disease progresses and some form of therapy becomes necessary

Radiation Therapy Roentgen ray therapy has been used in chronic lymphocytic leukemia for many years Therapy is most often directed to limited areas such as the spleen or lymph nodes because serious damage to the bone marrow may follow repeated spray irradiation In chronic lymphocytic leukemia the sensitivity of the nodes to roentgen therapy varies greatly from patient to patient. In some patients the nodes regress promptly but in others they remain refractory despite large doses

Radioactive phosphorus (P^{32}) which can be administered orally or intravenously is considered by many to be the best form of radiation therapy From 20 or 25 per cent of the administered oral dose is lost in the feces and a comparable amount of the intravenous dose is excreted in the urine.[1] The recommended dose of radioactive phosphorus is 0 1 millicurie per kilogram of body weight, given in divided doses of 1 millicurie per day[6] Excellent results have been reported from therapy of this type administered regularly even when the patient is asymptomatic

Alkylating Agents. The alkylating agents have a prominent place in the therapy of patients with chronic lymphocytic leukemia. The drugs that are used most widely at the present time are triethylenemelamine (TEM) and *p* (di 2-chloroethylamino) phenylbutyric acid (chlorambucil, CB 1348 Leukeran)

TEM is initially administered in a dose of 2.5 to 5 0 mg before breakfast and subsequent doses are adjusted to the needs of the patient In our experience it has been difficult to treat patients with chronic lymphocytic leukemia with TEM because some are extremely sensitive to small doses of the drug Because of the variable response obtained with TEM the initial dose should be small and the patient carefully observed for evidence of bone marrow depression over a period of two to three weeks before a second dose is administered. Bethell recommends an oral dose of 2.5 mg of TEM with 2 gm of sodium bicarbonate by mouth two hours before breakfast for one two or three consecutive days depending upon the extent of the disease as estimated from the leukocyte count adenopathy splenomegaly and bone marrow involvement[7] Treatment is then withheld for four weeks when another dose of 2.5 mg. is usually administered. Some patients are given additional doses of 2.5 mg. at intervals of four weeks or longer while others are treated in a more symptomatic fashion.

Chlorambucil is administered in doses of 0 1 to 0.2 mg. per kilogram of

dence of the disease. Visual disturbance due to retinal hemorrhages is at times the initial complaint. In some patients the initial symptoms are those due to anemia—dyspnea on exertion, dizziness, palpitation, easy fatigue and weakness.

The most striking abnormality on physical examination is splenomegaly. Enlargement of the spleen is present in nearly all patients with chronic myelocytic leukemia at the time of the initial examination. In one series of patients the lower border of the spleen extended below the umbilicus at the time of the initial visit in 70 per cent.[6] As the disease progresses the splenomegaly responds less to treatment and sometimes steadily increases in size until it eventually fills the entire left half of the abdomen and occasionally the right lower abdomen as well. The sudden appearance of hernia or uterine prolapse has been noted in some patients with such massive splenomegaly.[8] In most of the patients the splenomegaly is associated with marked enlargement of the liver. The liver is usually firm and non tender. Mild degrees of enlargement of the superficial lymph nodes may be seen in patients with chronic myelocytic leukemia but the enlargement does not reach the degree seen in patients with lymphocytic leukemia. Frequently no significant lymphadenopathy is present. Retinal lesions such as engorgement of the veins and white centered areas of hemorrhage are seen in a high percentage of the patients with chronic myelocytic leukemia.[1, 17, 46] At times leukemic infiltration along the peripheral portion of the distended veins and blurring of the disc margin are present. The skin and mucous membranes are often pale and petechial lesions and ecchymoses are not unusual.[1] Light or moderate pressure on the lower portion of the sternum produces pain in many patients. This tenderness is present during the active phase of the disease and often subsides as the disease responds to treatment.

Roentgenologic examination may reveal osseous lesions in various parts of the skeleton. Subperiosteal new bone formation, osteolytic lesions and transverse bands of diminished density at the ends of the long bones are the commonest abnormalities.[0, 6, 94] Osteosclerotic lesions are less common. Rarely the patient may develop acute pain in a joint because of involvement of the synovia by hemorrhage or infiltration with leukemic cells.[4]

Laboratory Manifestations

Anemia occurs at some time in practically all patients with chronic myelocytic leukemia but is not invariably present when the patient is first seen. The anemia tends to increase as the disease progresses and improves as the disease regresses in response to treatment. The anemia is usually normocytic and normochromic. The hemoglobin may be as low as 4 or 5 gm per 100 ml but is more often in the range of 7 to 9 gm per 100 ml when the patient is first seen. The reticulocyte count is usually normal or slightly elevated. The anemia may be the result of abnormal bleeding. In addition anemia may occur as the result of diminished erythropoiesis secondary to the encroachment on the erythropoietic tissue of the marrow by the leukemic cells and recent studies have shown that the erythrocytes of some patients

spleen was barely palpable and the leukocyte count was 12 000 per cu mm Since this course of treatment the patient has felt well and has worked full time for the past 11 years On several occasions while the patient remained asymptomatic the leukocyte count rose from 20 000 to 130 000 per cu mm and then returned to the lower level without treatment In June 1956 the spleen again increased in size until it extended to the level of the umbilicus Following another course of irradiation to the splenic area the spleen edge receded to the left costal margin In the three years since that time the leukocyte count has varied between 16 000 and 20 000 per cu mm

Comment This case illustrates the commonly observed onset of chronic lymphocytic leukemia in later years and the relatively benign course which the disorder follows in some patients Over a period of 11 years irradiation of the spleen on but two occasions has controlled his disease satisfactorily Many patients with chronic lymphocytic leukemia survive for the normal life span and die from causes other than leukemia

CHRONIC MYELOCYTIC LEUKEMIA

Incidence

Chronic myelocytic (myelogenous) leukemia is found in all races and in both sexes and is primarily a disease of middle age In 1924 Minot Buchman and Isaacs[6] presented data on 130 patients with chronic myelogenous leukemia In this series the commonest age of onset was from 35 to 45 years Fifty six per cent of the patients were males In 1951 Shimkin Mettier and Bierman[91] reviewed the incidence and distribution of chronic myelogenous leukemia compared their data with those of Minot Buchman and Isaacs and noted an increased incidence of the disease particularly in women and an increased age at onset

Clinical Manifestations

The onset of chronic myelocytic leukemia is usually unobtrusive At times the presence of chronic myelocytic leukemia is discovered during a routine examination[103] The period between the appearance of symptoms and the time the patient seeks medical advice is usually from eight to nine months The most common initial symptoms are the presence of discomfort in the left upper quadrant or the upper abdomen or the accidental finding of a mass in this area The patient often complains of a sensation of fullness and gaseous eructations after eating Not infrequently signs of active disease such as loss of weight fever and night sweats are present Usually the persistence and progression of these relatively mild symptoms lead the patient to seek medical advice

Less often the disease begins more abruptly Massive gastrointestinal hemorrhage manifested by either hematemesis or melena may be the first definite indication of disease Other individuals seek medical advice because of aching in the back and extremities or because of a pleuritic type of pain in the left upper quadrant and lower portion of the left chest The occurrence of hemorrhages or infiltrative lesions in the skin may be the first evi

with this disease have a shortened survival time which indicates that an element of hyperhemolysis is present

The most striking abnormality in myelocytic leukemia concerns the leukocytes The total leukocyte count in different patients ranges from less then 1000 per cu mm to more than 1 000 000 per cu.mm The leukocyte count is commonly between 100 000 and 300 000 per cu.mm Such high values are the result of an increase in cells of the granulocytic series segmented neutrophils band cells juveniles and myelocytes A few myeloblasts and promyelocytes may be present but they do not constitute a large percentage of the cells in chronic myelocytic leukemia The eosinophils and basophils are usually increased. When the cells are stained for alkaline phosphatase activity very few positive cells are found In a series of patients with known granulocytic leukemia studied by the authors less than 3 per cent of the segmented and band forms showed appreciable evidence of alkaline phosphatase activity This finding is in agreement with the results obtained previously by others[1,2]

The platelet count of the patient with chronic myelocytic leukemia is characteristically increased Platelet counts over 1 000 000 cu mm are sometimes seen. However when chronic myelocytic leukemia begins with a picture that resembles aplastic anemia thrombocytopenia is often present

Examination of the bone marrow usually reveals a marked increase in cellularity that is due chiefly to an increase in cells of the myeloid series Often the percentage of the younger cells in this series is increased but at times the differential count of the marrow falls within normal limits Even in such circumstances the marked increase in the number of cells of the granulocytic series leaves little doubt about the diagnosis Erythropoiesis is normoblastic in type and megakaryocytes usually are present in normal or increased numbers

Other laboratory findings in this disease include the following elevated basal metabolic rate with a normal radioactive iodine uptake [6] and elevated uric acid level

Clinical Course and Prognosis

The average survival time of patients with chronic myelocytic leukemia from the time of the onset of symptoms to death is usually about three or four years[6, 91] The five-year survival rate is about 15 to 20 per cent,[91] and occasionally patients live for 15 to 20 years The initial course of treatment may be followed by an excellent clinical remission but relapse inevitably occurs The development of relapse is usually manifested by loss of weight fever night sweats increasing anemia increasing size of the spleen and the recurrence of sternal tenderness The leukocyte count usually rises during

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Figure 55 Chronic lymphocytic leukemia

Figure 56 Chronic myelocytic leukemia.

From H Heyer L. and Begemann H Atlas der klinischen Hamatologie und Cytologie. Berlin-Gottingen Heidelberg Springer 1955

missions and the patient ultimately becomes refractory to further treatment of this type The major disadvantages of this form of therapy are the necessity of the patient's being near a hospital where the therapy can be administered and the not infrequent occurrence of radiation sickness with anorexia nausea, and vomiting

Radiation in the form of P^{32} has been used successfully in the treatment of chronic myelocytic leukemia Radioactive phosphorus administered orally or intravenously is selectively concentrated in the rapidly dividing cells of the bone marrow and the leukemic tissue It has a half life of 14 3 days and emits soft beta radiation that penetrates only a few millimeters in tissue[31] The dose must be individually determined and depends on the size of the patient, the amount of marrow involvement and the extent of the disease elsewhere The usual initial dose varies from 4 to 8 millicuries A second smaller dose 2 to 4 millicuries is given if the disease has not been controlled in six to nine weeks[66] After the disease has been brought under control the patient returns for periodic visits at intervals of from three to six months[7] Osgood on the other hand feels that the patient should be treated as soon as the diagnosis is made An initial dose of 0.5 to 2 5 millicuries of P^{32} is given intravenously and an effort is made to keep the disease under control by a plan of "titrated therapy that consists of small individual doses of P^{32} at intervals of from four to twelve weeks[67] Osgood reports that results with this technique are superior to those obtained with other therapeutic regimes in that patients so treated survive longer and spend about 85 per cent of their remaining life span at their usual activities

Chemotherapy Among the earlier treatments used for chronic myelocytic leukemia were the chemotherapeutic agents arsenic and benzol. These are used rarely if at all at the present time but newer chemotherapeutic agents have assumed an important place in the management of this disease Among the most important of these are Myleran (1-4 dimethanesulfonyloxy butane) urethan (ethyl carbamate) Purinethol (6-mercaptopurine 6MP) TEM (triethylenemelamine) and colcemide (deacetylmethylcolchicine) All of these agents can produce serious bone marrow depression and for this reason blood counts at regular intervals are a necessary part of the routine when these drugs are employed

MYLERAN is considered by many to be the drug of choice in the treatment of chronic myelocytic leukemia at the present time It was developed at the Chester Beatty Institute during studies undertaken to develop alkylating agents other than nitrogen mustard[7] The use of this drug was initially reported by Galton[3] and the good results that he reported have been amply confirmed[10, 45, 6, 7, 61] The initial dose of the drug for the average adult is usually 2 mg once or twice a day When the leukocyte count has fallen to the range of 10 000 to 20 000 per cu mm the drug may be discontinued until a relapse occurs Maintenance therapy of 2 mg per day or 2 mg two or three times a week is preferred by some authors[7]

TEM another alkylating agent is also effective in the treatment of chronic myelocytic leukemia The drug has been administered in courses

relapse but clinical relapse does not invariably follow an increase in the leukocyte count If the leukocyte count continues to rise over a period of several months a relapse is likely Subsequent courses of therapy generally bring remissions of shorter duration and the response of the spleen and blood is usually not nearly so satisfactory as in the first remission As the disease progresses splenomegaly and anemia become more or less constant features The course of this disorder is sometimes accompanied by severe attacks of left upper quadrant pain and fever which are the result of splenic infarction or perisplenitis A transformation into a myeloblastic leukemia is a common cause of death [67] The terminal stages are usually characterized by cachexia persistent fever, night sweats the persistence of anemia and the occurrence of hemorrhages The final episode is usually either a bacterial pneumonia or a fungus infection At times intracranial or gastrointestinal hemorrhage is the terminal event

Treatment

Though chronic myelocytic leukemia may be discovered on a routine examination when the patient is asymptomatic it is rare that more than a few months will elapse before the patient will need some form of treatment Occasionally in such circumstances several years elapse before symptoms occur [103] Both supportive and more specific measures are employed in the treatment of chronic myelocytic leukemia Specific treatment may have one or two objectives Treatment may be aimed at reducing the total extent of the disease in the hope that many of the manifestations of the disease will improve or treatment may be more restricted in which case it is considered as a symptomatic measure directed toward more localized manifestations of the disease

The most important supportive measures are the use of transfusions and the use of antibiotics Anemia is treated by transfusion if it is severe or if it fails to respond as the disease improves with specific therapy Antibiotics are used to treat infections when present however the possible emergence of resistant bacteria or fungus infection makes their prophylactic use danger ous Patients with leukemia are prone to infections and all patients who present with what appears to be a febrile relapse of leukemia should be studied carefully for some superimposed infection such as pneumonia or pyelitis that may be treated successfully

Radiation Therapy Roentgen therapy first used by Pusey in 1902 [8] was for many years the most widely used form of treatment for chronic myelocytic leukemia Programs of therapy have varied both in the area of the body irradiated and in the amount of irradiation given Irradiation is most often directed towards the spleen in single treatments of 100 r A total of 600 to 1200 r is given over a period of several weeks Such treatment usually produces a diminution in the size of the spleen a decrease in the leukocyte count and improvement in the anemia The initial remission usu ally lasts from six to nine months Subsequent therapy produces shorter re

In October 1955 she was hospitalized because of a fever of 105 F roentgenographic evidence of an infiltrate or exudate in the hilar regions of both lungs hepatomegaly splenomegaly and a leukocyte count of 59 000 per cu mm The fever and pulmonary abnormalities persisted unchanged for four weeks despite numerous antibiotics Myleran was then administered orally in a dose of 4 mg each day The fever declined gradually and after three weeks the patient was afebrile At this time the leukocyte count was 10 000 per cu mm and a roentgenogram revealed clearing of the lung densities The patient continued active and felt well on a maintenance dose of Myleran 2 mg two or three times a week for about two years

In September 1957 it became necessary to give transfusions every few months because of recurrent anemia Myleran was discontinued even though the bone marrow examination at this time revealed greatly increased granulocytic activity associated with hypoplasia of the erythroid series Several examinations disclosed no reticulocytes in the peripheral blood In November 1957 the leukocyte count had risen to 90 000 per cu mm and the majority of the cells in the peripheral blood were myeloblasts Because of the predominance of blast cells 6-mercaptopurine was administered in a daily dose of 2 5 mg per kilo The patient again responded well at the end of one month the leukocyte count was 6000 per cu mm and the myeloblasts had almost completely disappeared from the smear Following a remission of several months relapse occurred and the disease was refractory to further treatment of all kinds In July 1958 six years after the initial symptoms the patient died with cachexia anemia and pneumonia

Comment The onset of the disease in this patient at middle age with vague symptoms of weakness abdominal discomfort, splenomegaly anemia and leukocytosis is the common one At various times she had clinical remissions with the usual methods of therapy—roentgen ray radiophosphorus and Myleran As is often the case severe refractory anemia and the blood picture of myeloblastic leukemia developed in the later stages At this time a brief remission followed the use of 6-mercaptopurine In the terminal stage the disease was refractory to all measures and a fatal infection occurred

MONOCYTIC LEUKEMIA

Monocytic leukemia was first described as a distinct clinical entity by Reschad and Schilling Torgau in 1913 They reported a 33 year old patient who gave a history of exhaustion inflamed gums and loss of appetite of six weeks duration during the week prior to admission chills and epistaxis occurred Physical examination disclosed marked pallor a papular rash, ulcerated bleeding gums and retinal hemorrhages The erythrocyte count was 2 2 mil per cu mm and the leukocyte count was 15 000 per cu mm Seventy two per cent of the leukocytes were large mononuclear and transitional cells many were large bizarre lobed cells with basophilic cytoplasm The patient died in coma twenty days later on the day prior to death the leukocyte count was 56 000 cmm and the erythrocyte count was 920 000 cmm Postmortem

that consist of from two to ten doses of 5 mg each or as a continuous therapy with doses of 5 mg at intervals that vary from one week to one month.[7] While good results can be obtained with TEM it has proved more unpredictable and less satisfactory than Myleran in our experience

URETHAN has been used with some success in the treatment of this disease It is administered in enteric coated capsules 0 3 gram three times a day after meals The dose is increased gradually to 3 0 or 4 0 grams per day When the leukocyte count has fallen to satisfactory levels the drug is stopped [3] Urethan although effective in some patients has been largely discarded because good results occur in only about one third of the patients [7] while anorexia nausea and vomiting are frequently troublesome

COLCEMIDE which blocks mitosis is sometimes useful in patients with chronic myelocytic leukemia who undergo a terminal myeloblastic transformation The initial dose of the drug is 4 mg daily divided into two doses morning and evening Larger doses are required in some patients The maintenance dose varies with different individuals but is usually about one third of the initial dose [7] The maintenance therapy is started after a three or four day rest period which is allowed when the white count drops to 30 000 per cu mm Although in general the response to this drug is less than with other forms of therapy it may be tried when radiation and the alkylating agents have become ineffective

6 MERCAPTOPURINE will induce remissions in some patients with chronic myelocytic leukemia but appears to be inferior to Myleran in this type of disease [13] However it may be an effective form of therapy should a myeloblastic transformation occur in the terminal phase of the disease [7] This drug is usually given orally in a single daily dose of 2 5 mg per kilo

Illustrative Case

A 43 year old white housewife enjoyed her usual health until the summer of 1952 when she first noticed increased fatigue irregular fever weight loss and vague abdominal discomfort In October 1952 she consulted her physician who found her to be moderately pale The spleen was palpable 2 finger breadths below the left costal margin The erythrocyte count was 3 mil per cu mm hemoglobin 9 5 gm per 100 ml leukocyte count 57 000 per cu mm Differential count (per cent) myelocytes 25 polymorphonuclears 52 lymphocytes 16 eosinophils 4 basophils 3

A diagnosis of chronic myelocytic leukemia was made and the patient was treated with roentgen irradiation of the spleen She responded well and a month later the leukocyte count had fallen to 19 000 per cu mm and the hemoglobin had risen to 11 5 gm per 100 ml During the next two years the patient remained active and was treated with roentgen therapy at intervals

Late in 1954 her symptoms increased and a hematologic relapse occurred Because of the decreasing effectiveness of x ray therapy she was treated with 8 1 millicuries of radioactive phosphorus in January 1955 Following this therapy the patient was asymptomatic for eight months



Figure 57 Hypertrophied gums in a patient with acute monocytic leukemia

cytic type of anemia.[18, 4] The leukocyte count is nearly always increased but a normal total count is sometimes seen [18, 96] The initial count is usually in the range of 15 000 to 45 000 per cu mm but counts as high as 400 000 per cu. mm are reported[14] counts of over 100 000 per cu mm are common in the terminal stages Cells of the monocytic series are increased and constitute from 30 to 90 per cent of the total Some appear to be normal adult monocytes but usually blast forms and promonocytes are present in addition there are usually large abnormal multilobulated cells that have folded nuclei and pseudopodia or vacuolation in the cytoplasm The peripheral blood often contains nucleated red cells the platelet count is usually reduced The bone marrow is hypercellular blast cells and older cells of the monocytic series predominate

Acute monocytic leukemia generally runs a rapid course that terminates within two or three months Some patients undergo a partial remission when treated with adrenocortical steroids the alkylating agents or 6 mercaptopurine but even in these patients life is rarely prolonged for more than six or eight months [96]

Illustrative Case This 41 year old woman was admitted to the obstetrical service of the hospital in the seventh month of pregnancy because of weakness easy fatigue and severe ulceration of the mucous membranes of the mouth

She had had an uneventful gestation until six to eight weeks prior to admission when she noted the onset of mild migratory joint pains Four weeks before admission the left elbow became swollen and extremely painful She was admitted to another hospital where the physical examination revealed only a swollen left elbow with increased heat and the uterus enlarged to a size compatible with a six month pregnancy Laboratory

examination revealed splenomegaly and infiltration of the marrow skin and liver with large mononuclear cells The authors believed that the case represented a pure type of acute large mononuclear and transitional cell leukemia that was distinct from myeloid and lymphatic leukemia

Although the existence of a third cell type of leukemia was questioned by hematologists who considered the reported cases to be only variant of the other types of leukemia the occurrence of a monocytic type of leukemia is now accepted generally The earlier cases that were reported were instances of the more common acute form of the disease the chronic form was not described until 1934 by Doan and Wiseman ~

Monocytic leukemia is less common than myelocytic and lymphocytic leukemia It has been reported to constitute from 2 to 16 per cent of all leukemias [4, 8] Apparently the diagnosis is made more frequently when supravitally stained preparations are studied than when fixed smears stained with Wright s stain alone are examined The chronic form of the disease has been variously defined as that in which the patient survives for six months and twelve months after onset of symptoms About 25 or 30 per cent of the patients with monocytic leukemia have the chronic form of the disease [4, 78, 96]

Acute Monocytic Leukemia

The acute form of the disease may be present from the onset or may occur as the terminal episode in patients with the chronic form Males are affected more often than females the proportion of males in one series being 65 per cent [4] A mean age of 44 years was found in one series [4] but the ages of the reported patients have varied from 11 months to 83 years [96]

The first clinical manifestation of the disease is usually the gradual onset of weakness and easy fatigability but in some patients there is an acute onset with high fever abnormal bleeding and prostration Physical examination generally reveals pallor and slight generalized enlargement of the lymph nodes The spleen is palpable in about three fourths of the cases [8] the lower border usually extends from 1 to 4 cm below the left costal margin but enlargement of the spleen may not be detected clinically even in the most fulminant cases [96] Hepatomegaly is usually present As was first emphasized by Forkner often the most striking abnormalities on the physical examination are found in the gums and buccal mucosa [8] Relatively early in the disease the gums are swollen and pale and later they become ulcerated and necrotic When such lesions are present they are usually accompanied by moderate enlargement of the cervical lymph nodes Gingival hyperplasia and other lesions of the oral cavity are seen occasionally in other types of acute leukemia but they are much more common in the monocytic type where the frequency may be about 75 per cent (Fig 57) [96] Anal fissure sometimes associated with perirectal abscess is found often enough in patients with this disease to warrant a special search for these lesions [96] Involvement of the skin in the form of erythematous papular or infiltrative lesions occurs but is not common

Laboratory examinations generally disclose a severe progressive normo

At postmortem examination there was enlargement of the liver spleen lymph nodes and kidneys The bone marrow was entirely replaced in the axial skeleton and partially in the femur by the leukemic cell which was a large mononuclear form with a moderate amount of dark cytoplasm and a notched or folded nucleus that contained two or three small nucleoli and a moderately dense chromatin Microscopic leukemic infiltrates were present in the interstitial tissues of nearly all organs and appeared as gross nodules in the kidneys The uterine cervix was heavily infiltrated by the leukemic cells as well as by hemorrhage The uterine cavity contained a necrotic decidual lining in which there were many colonies of bacteria and bacteria had been disseminated throughout most of the other tissues The kidneys showed the lesions of lower nephron nephrosis The axial bone marrow contained broad areas of coagulative necrosis with some foci of acute hypoplasia and regeneration with reticulum cells

COMMENT This patient presents many of the features commonly found in patients with acute monocytic leukemia The onset of the illness with weakness easy fatigue and joint pain is not unusual in any type of acute leukemia but the early development of gingival hyperplasia and ulcerative stomatitis is more common in the monocytic type Identification of the cell line involved in acute leukemia is often difficult and is particularly so in the monocytic type It is not unusual for a large number of cells to be classified as belonging to the monocytic series on the initial examination while on subsequent examination the cells appear to belong to the granulocytic series Postmortem examination confirmed the clinical diagnosis of monocytic leukemia in this patient The effect of treatment is difficult to evaluate At least the patient survived long enough to be delivered of a viable infant Careful periodic examination of the child is indicated because the occurrence of leukemia in a nine months old infant born of a leukemic mother has been reported

Chronic Monocytic Leukemia

Chronic monocytic leukemia is predominantly a disease of middle age The ages of the reported patients have ranged from 17 to 68 but the mean age is about 56 the disease is unusual in persons less than 40 years of age[54 96] Males are affected more often than females In the reported series males have constituted from 75[4 96] to 84 per cent of the total[7]

This form of the disease usually has an insidious onset characterized by weakness and easy fatigue A history of repeated episodes of infection of the mucous membranes respiratory tract, and skin is common[96] Physical examination is usually normal except for pallor and evidence of weight loss Splenomegaly gingival lesions and skin lesions are less common than in the acute form of the disease all are often absent when the patient is first seen but splenomegaly and hepatomegaly frequently appear later during the course of the disease

An almost constant finding in these patients is anemia, which is often the

studies at that time revealed hematocrit 33 per cent erythrocyte count 3.3 mil per cu mm hemoglobin 9 gm per 100 ml platelets 81 000 per cu mm and leukocyte count 7200 Differential count (per cent) myelocytes ("monocytoid") 28 juvenile 2 bands 9, segmented 28 lymphocytes 28 monocytes 4 eosinophils 1 Many of the young forms contained Auer rods Examination of the aspirated bone marrow revealed 12 per cent blast cells and 56 per cent young monocytes of bizarre form

A diagnosis of acute monocytic leukemia had been made Over the next ten days the leukocyte count rose to 16 000 per cu mm and the patient developed hypertrophy of the gums and severe ulcerative stomatitis Therapy was then instituted with 6 mercaptopurine 75 mg daily by mouth Over the next ten days the leukocyte count fell to 3000 per cu mm the platelets to 40 000 per cu mm and petechiae appeared over the dependent portions of the body 6 Mercaptopurine was discontinued and the leukocyte count rose to 15 000 per cu mm and the petechiae disappeared though the platelet count persisted at 31 000 per cu mm The patient was discharged to her home under the care of her physician

While at home during the week prior to final admission she was weak nauseated and continued to have migratory joint pains The ulcerative stomatitis worsened and she began to vomit intermittently

Physical examination on admission revealed an acutely ill woman with fever of 101° and pulse rate of 124 There was marked pallor of skin and mucous membranes Several ecchymoses were noted over the lower legs There was a foul ulcerative stomatitis which was associated with marked hypertrophy of the gums There was moderate anterior and posterior cervical adenopathy The fundus was four fingerbreadths below the xiphoid Fetal heart rate was 140 per minute

Laboratory examination hematocrit 17 per cent leukocyte count 3 100 per cu mm and differential count (per cent) blast cells 6 promyelocytes 17 myelocytes 5 juvenile 0 bands 5 segmented 17 lymphocytes 46 monocytes 4 Platelets were 15 000 per cu mm The patient was treated symptomatically Transfusions of whole blood and packed cells over the next eight days brought the hematocrit to 35 per cent The leukocyte count rose gradually during this period to 12 000 per cu mm and the platelet count rose to 38 000 per cu mm The differential count remained essentially unchanged

Adrenocortical steroid therapy was started on the fourteenth hospital day The patient went into labor that night and was delivered of a viable premature female infant of 1400 gm Following delivery her condition deteriorated She continued to vomit and had several gastrointestinal hemorrhages Her leukocyte count decreased progressively over the next six days to 400 per cu mm and no platelets were seen on smear She expired seven days following delivery about three months after the appearance of the first symptoms of her disease

Microscopic examination of the placenta and umbilical cord revealed no evidence of leukemic infiltration

monocytic leukemia when the patient is seen in the "preleukemic" phase which probably occurs in nearly one half of the patients and may last for months or even years[98] The presence of the disease can sometimes be suspected in patients who appear to have aplastic anemia because of the presence of a hypercellular marrow with a "megaloblastoid" type of erythropoiesis The presence of unidentified young cells an increase in cells of the monocytic series in the peripheral blood or in the marrow the presence of giant platelets and the occurrence of splenomegaly should also arouse suspicion At times the diagnosis can be established definitely only after a prolonged follow up period and frequent examinations

When the patient presents with anemia splenomegaly and leukocytosis the diagnosis is usually not difficult because of the presence of increased numbers of cells of the monocytic series of various stages of maturity in the peripheral blood and bone marrow Even in this circumstance difficulty in classifying the leukemia arises occasionally In some patients with leukemia the initial examination of the peripheral blood reveals a large percentage of cells that appear to belong to the monocytic series associated with blasts and young cells of the granulocytic series In some patients this blood picture persists but in others later in the course of the disease only cells of the granulocytic series are seen Such cases which at necropsy have no evidence of hyperplasia of the reticulum have been classified as the "Naegeli type of monocytic leukemia to distinguish them from the "Schilling" type that is associated with hyperplasia of the reticulum Cases of the Naegeli type appear to belong to the chronic myelocytic group and the existence of such cases has been advanced as evidence that in some circumstances monocytes are derived from myeloblasts[98]

The treatment of chronic monocytic leukemia is unsatisfactory Irradiation and splenectomy are of no value the alkylating agents and the antimetabolic drugs usually produce but little if any benefit Transfusions are usually necessary for the anemia which is refractory to other measures

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Figure 58 Leukemia cutis Both infiltrative and hemorrhagic lesions are present

presenting complaint The anemia which is usually at least moderately severe is either normocytic or macrocytic Hemoglobin levels of 5 to 7 gm per 100 ml are common The anemia is accompanied by leukopenia early in the course of the disease in most patients and thrombocytopenia generally occurs sooner or later The granulocytes are usually reduced while the cells of the monocytic series are increased As the disease progresses the leukocyte count rises cells of the monocytic series increase and in the terminal stage the picture of acute monocytic leukemia occurs

The appearance of the bone marrow varies in different patients In some the marrow is hypocellular and erythrocytic hypoplasia is evident in others the appearance is that of erythrocytic hyperplasia associated with cells that have an abnormal appearance and somewhat resemble megaloblasts because of an increase in the parachromatin of the nuclei An increase in cells of the monocytic series is sometimes evident early but massive invasion by the cells usually occurs only late in the disease

The recognition of chronic monocytic leukemia is often difficult when the patient is first seen Not infrequently patients with this disease are misdiagnosed as aplastic anemia because of the occurrence of anemia associated with leukopenia and thrombocytopenia that is refractory to treatment other than transfusion Patients are also misdiagnosed as "hypersplenism" because of the association of splenomegaly with anemia or pancytopenia and an apparently active cellular marrow [98] In such patients even the histologic study of the spleen removed at operation may not disclose the correct diagnosis [96] It is usually not possible to make a definite diagnosis of chronic

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tory tests and special procedures such as a biopsy. The investigation is directed toward the "probable" diseases first and specific tests for "possible" but "improbable" disorders are deferred.

Enlarged lymph nodes which provide the focal point of the diagnostic study in certain patients occur in a number of different diseases. Probably the commonest causes are regional infections such as infected wounds, dermatitis, pharyngitis, sinusitis, and abscessed teeth. Lymphadenopathy also occurs with relatively benign systemic infections such as rubella, infectious mononucleosis, and viral hepatitis and with more serious infections such as tuberculosis, brucellosis, syphilis, fungus diseases, and tularemia. Enlargement of lymph nodes may be due to the presence of neoplastic disease such as metastatic carcinoma, leukemia, lymphosarcoma, Hodgkin's disease, reticulum cell sarcoma, and giant follicular lymphosarcoma. Mild degrees of enlargement occur in serum sickness and hyperthyroidism. Moderate enlargement occurs in disseminated lupus erythematosus and Boeck's sarcoid.

History

As is true with nearly all diagnostic problems, an accurate history is almost essential. Even the age of the patient is helpful information because the more benign systemic infections, such as rubella and infectious mononucleosis, are common in children and young adults but are rare in middle-aged and older persons. Both the duration of the adenopathy and the presence or absence of various symptoms help in deciding which of the possible diagnoses are more probable. The recent development of adenopathy does not exclude a neoplasm from consideration, but the persistence of enlargement for some months makes an acute infection unlikely. Localizing symptoms such as pain and tenderness in the gland, and evidence of associated inflammation, such as pharyngitis, favor infection. A history of weight loss, recurrent fever, night sweats, and pruritus suggests the presence of systemic disease, most often one of the lymphoma group.

Physical Examination

The physical examination, particularly the characteristics of the superficial nodes, generally gives helpful information. The distribution, size, consistency, and mobility of the nodes are important, as is the presence or absence of tenderness. Probably the most important observation is whether the adenopathy is generalized or localized. If the involvement is generalized, some systemic disease becomes the primary consideration and regional adenitis can be excluded. If the involvement appears to be localized, the presence of systemic disease is not excluded but the first consideration is given to a careful search for some associated disease, either infection or neoplasm, in the area drained by the enlarged lymph nodes. In any patient with enlarged nodes the size is estimated carefully and is best recorded in centimeters rather than in less accurate terms such as "pea sized" and "egg sized." An estimate of the size of the nodes gives some indication of the

Other Disorders of the Lymphoid Tissue and Bone Marrow

THE DIAGNOSIS OF THE PATIENT WITH ENLARGED LYMPH NODES

THE MOST ACCURATE clinical diagnoses are made either by histologic study of diseased tissue or by the demonstration of the presence of the agent responsible for the disease In clinical practice the physician usually begins his diagnostic examination with the history and physical examination With the recognition of some particular sign or symptom complex such as lymphadenopathy the physician proceeds with the differential diagnosis by an orderly consideration of the different diseases that are known to produce the manifestations that have been observed The probability of the presence of one or more of the diseases under consideration increases as other evidence is found that supports those particular possibilities This evidence is obtained by a systematic study of the patient that includes various labora

the level of the umbilicus or below it is probable that the patient has some chronic disorder such as a disease of the lymphoma group rather than an acute infection such as infectious mononucleosis or viral hepatitis

Laboratory Examination

Laboratory examinations sometimes establish the diagnosis in patients with large lymph nodes and at other times permit the exclusion of certain diseases from further consideration The routine studies of the blood such as the hematocrit, leukocyte count and differential count, are indicated By such means leukemia usually can be detected if present and the diagnosis of other diseases such as infectious mononucleosis can be provisionally established or excluded An increase in the granulocytes favors bacterial infection or Hodgkin s disease and an increase in "pathologic" lymphocytes favors infectious mononucleosis or a viral infection Monocytosis suggests Hodgkin s disease tuberculosis or brucellosis The presence of anemia indicates that the patient probably has more than a localized acute infection More definitive information is provided by specific examinations such as the heterophil agglutination test and agglutination tests for brucellosis and tularemia Skin tests for tuberculosis and fungus diseases are sometimes helpful. Repeated attempts may be necessary to demonstrate the presence of lupus erythematosus cells" In certain patients other studies such as the electrophoretic study of the serum proteins and aspiration of bone marrow to obtain material for culture and cytologic study are important

Roentgenographic Examination

Roentgenographic examinations often give valuable information about the nature of the process that is manifested by enlargement of the superficial nodes A roentgenogram of the chest is usually necessary in order to detect disease in the lungs and determine the presence of involvement of the nodes in the hilar areas or mediastinum The location number and appearance of the lesions in the lung may indicate the probability of tuberculosis fungus disease primary or metastatic carcinoma or other disease Details of the appearance and location of the involved nodes in the hilar areas and mediastinum may help to distinguish between Boeck s sarcoid and one of the lymphoma group Examination of the gastrointestinal tract may reveal the presence of a primary carcinoma that was first suggested by the presence of Virchow s node behind the origin of the left sternocleidomastoid muscle Radiologic evidence of diffuse infiltration of the stomach and hypertrophied rugal folds favors lymphosarcoma over Hodgkin s disease and other disorders that may present with superficial adenopathy Evidence of skeletal involvement indicates that the adenopathy is part of a serious disorder even though the roentgenographic appearance of the osseous lesion may not permit the differentiation between the several possibilities.

Lymph Node Biopsy

If the diagnosis is not made by other means biopsy of a lymph node is often necessary The decision as to the need for a biopsy is reached after an

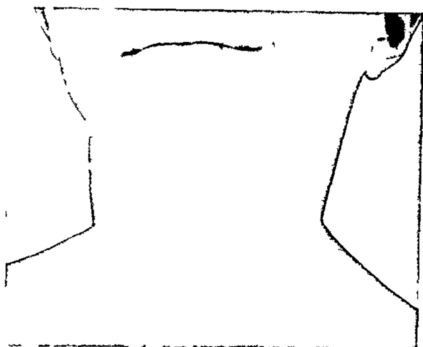


Figure 59 Enlargement of cervical lymph node in a patient with Hodgkin s disease

probability of the presence of certain diseases The nodes in hyperthyroidism and serum sickness are rarely more than 0 5 or 1 0 cm in diameter and in disseminated lupus they seldom measure more than 1 0 to 3 0 cm In the *diseases of the lymphoma group the nodes are sometimes greatly enlarged* Careful estimations of the size of the nodes and the constancy of the size are helpful in following the progress of the disease while the patient is under observation The presence of tenderness in a node usually indicates that infection is present but marked tenderness also occurs in the nodes of some patients with other diseases particularly Hodgkin s disease Fixation of a node to the skin or deeper tissues indicates that the infection or neoplasm has involved the surrounding area. Sinus tracts or scars over an area of adenopathy suggest tuberculosis or fungus disease A relatively small node that is stony hard in consistency suggests metastatic carcinoma, but extremely hard nodes that measure several centimeters in diameter also occur with calcification in sarcoma and in tuberculosis In diseases of the lymphoma group characteristically the nodes are moderately firm rubbery and somewhat resilient but many exceptions occur The normal bean *shaped contour of the node is more apt to be maintained with infection than* with neoplasm

Other details of the physical examination are also important The detection of a *focus of infection in an area related anatomically to the adenopathy* usually warrants a diagnosis of regional adenitis The presence of splenomegaly and hepatomegaly suggests some generalized infectious process or a disease of the lymphoma group *If the spleen is firm and extends halfway to*

tinguished from each other by their clinical manifestations An accurate diagnosis is made only by microscopic study of a specimen of involved tissue usually a lymph node Although of unknown etiology the diseases are generally considered to be neoplastic in nature

The term malignant lymphoma is a convenient one for various reasons In clinical practice it provides a designation for a provisional diagnosis until a more accurate one can be made In addition the histologic study of biopsy material sometimes makes it possible to place a disorder in this group of diseases even though a more precise diagnosis cannot be made with certainty In this circumstance the patient often can be treated properly without benefit of an accurate diagnosis because the same principles of therapy and the same therapeutic agents are used in the management of the different diseases It is convenient to group these diseases for the purpose of a clinical discussion because of the similarities in clinical manifestations methods of diagnosis treatment and prognosis

Hodgkin s disease is named after Thomas Hodgkin who first described the disorder in 1832 [3] In 1893 Kundrat recognized lymphosarcoma as a disease entity different from leukemia [3] Giant follicular lymphoma sometimes called "Brill Symmers disease" was described by Brill Baehr and Rosenthal in 1925 and by Symmers in 1927 [6] Roulet has been given credit for first clearly identifying reticulum cell sarcoma as a distinct type of tumor [49]

CLASSIFICATION

Numerous classifications have been proposed for this group of diseases that involve the lymphoid tissue primarily [4, 1, 16, 30, 48, 53, 8] The classification shown here which divides the lymphomas into four main groups is a useful one for clinical purposes

Classification of Malignant Lymphoma

1 Hodgkin s disease
2 Lymphosarcoma
3 Reticulum cell sarcoma
4 Giant follicular lymphoma (Brill Symmer disease)

Some idea of the relative frequency of these diseases is given by the diagnoses made in one series of 618 cases of "lymphoma" When classified according to the above system the various forms of Hodgkin s disease constituted 37 per cent lymphosarcoma 35 per cent reticulum cell sarcoma 20 per cent and giant follicle lymphoma 8 per cent [16]

Although a specific diagnosis can usually be made by the histologic study of involved tissue at times the pathologist may have difficulty in identifying the disease from the study of material obtained by biopsy Different nodes from the same patient have been diagnosed as different diseases and changes considered to be characteristic of Hodgkin s disease sarcoma and giant follicle lymphoma have been reported in the same lymph node [7] The apparent transition of giant follicle lymphoma into the other

evaluation of each patient individually In one situation it may be apparent at the initial examination that a biopsy should be done promptly in another circumstance it may be decided that biopsy can be deferred for further observation and the performance of other tests If an undiagnosed enlargement of the lymph node in an adult does not subside significantly within a few weeks biopsy is nearly always indicated

Although no fast rules can be made for the performance of the biopsy the observance of some simple guides is helpful to both the pathologist and the clinician Although the pathologist bases his diagnosis on the histologic appearance of the specimen it is helpful if pertinent clinical data are submitted along with the biopsy specimen It is important that the pathologist receive representative tissue that is suitable for study If more than one node is enlarged the selection of the one to be biopsied is important In general cervical or supraclavicular nodes are preferable to axillary nodes nodes from all of these sites are preferable to nodes from the femoral area because the latter often have undergone alteration as a result of previous infection in the lower extremities When the patient has a disease that involves the lymph nodes without significant enlargement of the superficial lymph nodes frequently the diagnosis can be established by a supraclavicular biopsy particularly if pulmonary or mediastinal involvement is present Whenever possible the biopsy is performed and the diagnosis established before the patient receives any specific therapy because both irradiation and chemotherapy *can so change the appearance of the node that histologic diagnosis is difficult or impossible* Even careful handling of the node at the time of operation is important because trauma can distort the histologic appearance of the lesion When the diagnosis is uncertain and infectious processes do not seem to be excluded with reasonable certainty it is always advisable to send part of the node for histologic examination and part for bacteriologic study Unless this is done even tuberculosis or a fungus infection may pass unrecognized If the biopsy of a node does not provide a definite diagnosis a second biopsy may be indicated if no suitable node is available at the time periodic examination for the appearance of such a node is advisable

MALIGNANT LYMPHOMAS

Lymphoma, Lymphoblastoma

Malignant lymphoma is a term used to designate a group of diseases—Hodgkin s disease lymphosarcoma reticulum cell sarcoma and giant follicle lymphoma—that have certain features in common These diseases which involve mainly the lymphoid structures of the body cannot be dis

ness loss of weight and fever [0, 9] Cough dyspnea and pruritus occur less often but are not rare

Fever occurs sometime during the course of the disease in nearly every patient with Hodgkin's disease The fever may be continuous intermittent or cyclic An irregular type of fever that rises to 100 to 103 F at night occurs frequently and generally signifies that the disease is not confined to the superficial lymph nodes The well known "Murchison" or Pel Ebstein type of fever occurs in only a minority of patients [0, 7] In this syndrome the temperature curve shows successive daily rises over a period of about a week until it reaches a peak of 103 to 10o then subsides gradually to normal *After an interval of ten days to two months another febrile episode appears* The periods of remission gradually shorten unless the course of the disease is changed by therapy When this type of fever occurs it generally signifies mediastinal or intra abdominal disease A remittent type of fever similar to that seen in septicemia occurs in some patients with the acute fulminating type of Hodgkin's disease even in the absence of demonstrable infection

The variety of clinical manifestations that Hodgkin's disease may produce has been emphasized [9, 4] Pruritus or exfoliative dermatitis may be the first manifestation of the disease Disease in the intrathoracic region may present as an asymptomatic mediastinal tumor found on the roentgenogram or with evidence of the superior mediastinal syndrome (Fig 60) Intrathoracic

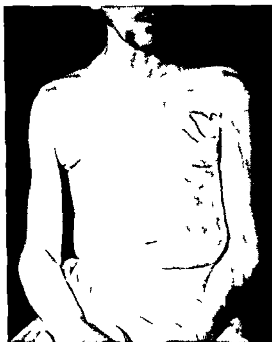


Figure 60 Infra red photograph demonstrating collateral circulation in a patient with *Hodgkin's disease and superior vena caval obstruction*

diseases in the group is well known[1] In one series of 84 patients with malignant lymphoma biopsies were repeated after intervals that varied from one month to 15 years (average 2 3 years) and a histologic diagnosis different from the initial one was made in 23 per cent in most instances the lesion had become less differentiated in appearance[16] In another series a transition from the biopsy material was found in 39 per cent of the autopsied cases in over one half of the autopsies lesions of more than one of the lymphoma group occurred[1] Although many pathologists consider that the histologic features of these diseases with the exception of giant follicle lymphoma remain relatively constant the inconstancy that has been noted *in some cases has led to the suggestion that the whole lymphoma group is* but a single neoplastic entity that has a number of variants[12]

CLINICAL FEATURES

I Hodgkin s Disease

The extensive literature on Hodgkin s disease that appeared prior to 1948 has been the subject of several excellent reviews[4 8] The clinical manifestations histologic appearance methods of diagnosis and variable natural history of the disease are well established Despite the numerous attempts that have been made to solve the problem the cause (or causes) of Hodgkin s disease has not been established A recent review indicates that the data suggestive of a viral etiology are increasing[6 33] Although the occurrence of Hodgkin s disease in association with other diseases notably tuberculosis and fungus infections has suggested more than a chance association all efforts to establish tuberculosis the avian tubercle bacillus diphtheroids and other bacilli and fungi as causes of Hodgkin s disease have proved unsuccessful[6 4 33]

The varied clinical manifestations of Hodgkin s disease may appear at any age and in either sex The disease occurred most frequently in the third decade in one series[1] but in another study an even distribution was found in the first seven decades[8] Males are affected more commonly than females the ratio being about two to one[16 7 1]

The clinical manifestations of Hodgkin s disease are varied because virtually any organ in the body may be involved by the disease process The incidence of lesions in various locations noted either during life or at autopsy in two series that totaled 485 patients[0 9] are as follows lungs 33 per cent mediastinum 33 per cent liver and spleen 50 per cent and 70 per cent skin "common" and 38 per cent bones 20 per cent and 7 per cent Involvement of the nervous system occurred in 12 per cent of another series[3] The disease is rarely found in the tonsils and gastrointestinal tract

Although nearly any sign or symptom may be the presenting one the commonest *initial symptom is painless enlargement of one or more lymph* nodes An initial manifestation of this sort usually in the cervical nodes occurs in about 85 per cent of patients with Hodgkin s disease[0 9] The next most frequent symptoms are pain in the back abdomen or chest weak

splenomegaly anemia and other abnormalities in the peripheral blood are the principal manifestations of the disease

Abnormalities that are demonstrable by physical examination depend on the presence of the disease in various organs or systems in different parts of the body Most commonly the lymph nodes spleen and liver are involved The degree of lymphadenopathy which is usually greatest in the cervical region varies from slight to marked The nodes are usually firm and rubbery in consistency but may be either soft or stony hard They are rarely tender and usually are not fixed to the skin or underlying structures Occasionally the size of the nodes especially in the cervical and submandibular areas varies spontaneously from day to day Palpable splenomegaly occurs in about from 50 to 70 per cent of patients at some stage of the disease [30] The degree of enlargement found in different patients varies from a barely palpable spleen to one that nearly fills the entire left half of the abdominal cavity usually the lower edge of the spleen is above the level of the umbilicus Less commonly the examination reveals evidence of ascites pleural effusion pericardial effusion superior vena caval obstruction and neurologic involvement

Laboratory examinations often disclose abnormalities particularly in the peripheral blood Normocytic anemia of some degree occurs in nearly all patients at some time during the course of the disease The anemia is usually the result of diminished erythropoiesis but in some patients increased hemolysis is the important factor The total and differential leukocyte counts are often normal early in the disease but a transitory increase in lymphocytes sometimes occurs An increase in blood platelets and an absolute increase in monocytes have been reported to be constant throughout the disease [8] In the more advanced stages of the disease leukocytes counts often rise to levels of 15 000 to 20 000 per cu mm or more The leukocytosis is associated with an increase in cells of the granulocytic series and a decrease in lymphocytes [8] Eosinophilia of considerable degree occurs in some patients Toxic granulation is often prominent and large bizarre platelets are often present The erythrocyte sedimentation rate is usually elevated when the disease is active The electrophoretic pattern of the serum proteins may reveal a reduction in albumin associated with an increase in globulin in the moderately advanced cases Abnormalities in the proteins similar to those found commonly in multiple myeloma sometimes occur in Hodgkin s disease when there is an increase in plasma cells [1 39] Reed Sternberg cells can be found in bone marrow smears only rarely [4] but the diagnosis has been made in 25 per cent of patients when aspirated bone marrow was studied in paraffin sections [4]

2 *Lymphosarcoma and Reticulum Cell Sarcoma*

Lymphosarcoma and reticulum cell sarcoma occur at any age but the highest incidence is found in the fifth and sixth decades [16 c] The incidence *in males is about twice that in females* [16 f]

The first evidence of disease is usually visible and palpable enlargement

disease may also be responsible for an unproductive cough dyspnea, or pleural effusion The first evidence of the disease sometimes appears in the abdominal region in the form of a retroperitoneal tumor splenomegaly or hepatomegaly Skeletal involvement which is most likely to occur in the vertebrae is usually associated with pain that may be present for weeks before an osseous lesion is demonstrable in the roentgenograms The presence of disease in the nervous system most often becomes evident because of pressure on the spinal cord or the fifth sixth seventh eighth or ninth cranial nerve [3] When involvement of the brain occurs it is generally associated with disease of the cranial bones The epidural tumor that causes cord compression is nearly always an extension from a lesion in a vertebra or from the adjacent mediastinal or retroperitoneal lymph nodes [3] Peripheral neuritis in the lower extremities is a troublesome symptom in some patients [39] It appears likely that the attacks of herpes zoster which are fairly common are caused by involvement of the ganglia by the herpes virus rather than by Hodgkin's disease because the herpetic lesions disappear spontaneously without treatment (Fig 61) Hodgkin's disease is often found to be the cause of a persistent fever of undetermined origin In still other patients



Figure 61 Herpes zoster in a patient with Hodgkin's disease

more frequently in reticulum cell sarcoma than in lymphosarcoma[16, 30]

Abnormalities occur in both the peripheral blood and bone marrow of many patients with lymphosarcoma and reticulum cell sarcoma Lymphocytosis of the peripheral blood which is sometimes due partly to an increase in the large lymphocytes is seen more often in these disorders than in Hodgkin's disease Some patients who are diagnosed as having lymphosarcoma later develop the peripheral blood picture of lymphocytic leukemia Eosinophilia and polymorphonuclear leukocytosis sometimes occur but are less frequent than in Hodgkin's disease Hemolytic anemia of the auto immune type occurs in some patients and at times is accompanied by thrombocytopenia The antiglobulin (Coombs) test is positive in at least half of the patients with malignant lymphoma who develop severe hemolytic anemia[28, 39] in some reports on the association of auto immune hemolytic disease and lymphoma only patients with positive tests are included[46] Abnormalities in the serum protein such as moderate decreases in albumin and gamma globulin and moderate increases in the alpha 1 and alpha 2 fractions occur commonly the occurrence of myeloma type proteins is not rare[1] An increased percentage of lymphocytes and an increase in the number of large mononuclear or reticulum cells in the bone marrow suggest the diagnosis of lymphosarcoma or reticulum cell sarcoma When material obtained by aspiration of the marrow is studied by the paraffin section method a positive diagnosis may be made in about half the patients with reticulum cell sarcoma In lymphosarcoma and giant follicle lymphoma a positive diagnosis can be made less often in this way[4]

3 *Giant Follicular Lymphoma*

Giant follicular lymphoma (follicular lymphoblastoma Brill Symmers disease) is about two and a half times as common in males as in females and occurs most often in the fifth or sixth decade[16, 17] Less than 5 per cent of the cases occur in individuals under 20 years of age This disorder has a more benign course than the other members of the lymphoma group but its tendency to undergo transition to lymphosarcoma reticulum cell sarcoma Hodgkin's disease and lymphocytic leukemia is recognized.[1, 14, 16, 17, 57]

The most common initial manifestation is enlargement of the superficial lymph nodes or spleen The involvement of the nodes is usually more or less generalized but localized disease also occurs Enlargement of the mediastinal nodes may produce marked impairment of respiration Enlargement of the retroperitoneal mesenteric and mediastinal nodes is common The presence of the disease in these locations is often associated with ascites or hydrothorax and the frequent occurrence of chylous ascites has been reported[15] The incidence of splenomegaly in giant follicular lymphoma is reported to be 33 per cent[17] Fever cachexia and other constitutional manifestations are rare[1] Although anemia is said to be unusual until late in the disease[2, 17] the frequent development of hemolytic anemia thrombocytopenic purpura and leukopenia in patients with splenomegaly has been reported[14] Involvement of the skin nervous system and skeleton is rare.[16, 17, 53]

of superficial lymph nodes In one series of 196 patients the disease made its appearance in this way in 65 per cent[9] The cervical nodes were involved in 59 per cent and the axillary inguinal and abdominal nodes were each involved in about 12 per cent mediastinal involvement was much less common In 40 per cent of the patients the lymph node enlargement was confined to one area in 32 per cent it was generalized and in the remainder two or three regions were involved The involved nodes which are generally moderately firm and resilient are movable unless local invasion has occurred

Although lymphosarcoma reticulum cell sarcoma, and Hodgkin s disease cannot be distinguished from each other by the clinical manifestations in the individual patient the diseases follow somewhat different patterns Splenomegaly is less common in lymphosarcoma than in Hodgkin s disease (20 per cent compared to 50 to 70 per cent)[9] fever occurs less often[16] and anemia develops later The "Murchison Pel Ebstein" type of fever curve which was observed during the course of the disease in 16 per cent of one group of patients with Hodgkin s disease is rare in other members of the lymphoma group[16] Lesions of the gastrointestinal tract occur more commonly in lymphosarcoma and reticulum cell sarcoma than in Hodgkin s disease[16, 9, 40] The stomach small intestine or colon may be involved The gastric lesion most often consists of a diffuse infiltration that may be recognized on the roentgenologic examination of the stomach because of the presence of exaggerated, stiffened rugae and diminished peristalsis The most frequent site of disease in the small bowel is the terminal ileum where the lesion may produce bleeding or intestinal obstruction Involvement of the nasopharynx and tonsillar areas is rare in Hodgkin s disease but occurs oftener in lymphosarcoma Lesions of the nervous system and skeleton occur



Figure 62 Chest roentgenograms in a patient with lymphosarcoma showing mediastinal adenopathy and hydrothorax before therapy (A) appearance after irradiation (B)

In the majority of patients the distinction between lymphosarcoma and chronic lymphocytic leukemia is made without difficulty because of the increases in total leukocyte count and percentage of lymphocytes that are found in most patients with leukemia However in the occasional patient this differential diagnosis is not easy because there seems to be a close relationship or overlapping between the two diseases It is known that some patients who are diagnosed as having lymphosarcoma from a node biopsy at a time when the blood and bone marrow appear entirely normal later develop the hematologic features of lymphocytic leukemia The term "leukosarcoma" has been used to designate these transitional cases In some such patients the classic features of leukemia develop later but in others the abnormalities in the peripheral blood prove to be transient and at necropsy months or years later there is no evidence of widespread invasion of the various organs that usually occurs in leukemia [39] In still others the abnormalities in the blood, such as a mild absolute lymphocytosis persist but a frankly leukemic picture does not develop

Management of the Patient with Malignant Lymphoma

It is important to establish a diagnosis histologically before treatment is started, and unless the biopsy suffices at least to place the diagnosis in this group of diseases treatment is best deferred, except in unusual circumstances The decision regarding the type and amount of treatment must be based on a careful evaluation of the individual patient which takes into consideration the condition of the patient as well as the type extent and location of disease An evaluation of this sort is important because any treatment can prove deleterious or even fatal to the patient if given injudiciously Regular periodic follow up examinations constitute an important part of the therapeutic program Not only do these visits provide the best means for the physician to follow the course of the disease but they can be an important part of the supportive treatment The psychologic aspect of the management becomes even more important when the disease is relapsing or progressing despite treatment A moderately aggressive and hopeful attitude toward therapy is most likely to produce the best results at any stage of the disease and is usually the best attitude for the patient s morale

The same therapeutic agents are employed in the treatment of patients with the different diseases of the lymphoma group and the same general plan of therapy can be followed in each disease In the treatment of diseases that have such varied clinical manifestations it is important to have a basic plan of therapy that can be followed through the variety of different symptoms that may develop In formulating or following a plan for a patient with any disease of the lymphoma group we have found it very convenient to classify the patient according to the severity or extent of the disease as Peters recommends for Hodgkin s disease [44] Such a classification shown herewith is also helpful in estimating the prognosis in an individual patient.

1 *Mycosis Fungoides*

Mycosis fungoides is a form of malignant lymphoma of the skin At varying stages the lesions may be erythematous exfoliative infiltrative with nodules or plaques or atrophic with ulceration Although the prognosis is generally serious for those with this disorder, patients with localized lesions sometimes remain essentially well for decades and die from some other cause As a rule the disorder terminates as one of the malignant lymphomas[43] particularly reticulum cell sarcoma[10]

DIAGNOSIS

The presence of one of these diseases is suggested by the clinical manifestations but the antemortem diagnosis can be made only by biopsy A suitable superficial node is usually available Preference is given to cervical supraclavicular and axillary nodes femoral and inguinal nodes are used only if no other is suitable When none of the superficial lymph nodes is significantly enlarged the diagnosis can sometimes be made by a supraclavicular biopsy particularly if disease is present in the lung or mediastinum At times the diagnosis can be made by the biopsy of a local tumor mass or by needle aspiration of an enlarged liver Study of stained smears made from material obtained by needle aspiration of a lymph node is also a helpful procedure in experienced hands[41] The diagnosis can be made only rarely by a study of stained smears made from aspirations of the bone marrow[3, 7] but examination of either a surgical biopsy of the marrow or paraffin sections made from aspirated material is more likely to yield the diagnosis Thus type of examination of the bone marrow may be a very helpful procedure when the diagnosis is obscure[4] Cell blocks made from pleural or ascitic fluid rarely provide a positive diagnosis in diseases of the lymphoma group[39]

A negative" report of a biopsy does not mean that the presence of one of these diseases has been excluded If the pathologist can make no diagnosis on the specimen other than lymphoid hyperplasia or "hyperplastic lymphadenitis another biopsy should be performed If no suitable nodes are available the patient should be examined periodically in the expectation that a lesion suitable for biopsy will develop later

Hodgkin s disease lymphosarcoma reticulum cell sarcoma and giant follicle lymphoma must be distinguished from other diseases that may produce similar clinical manifestations The possibility of one of the lymphoma group is suggested by enlargement of superficial lymph nodes splenomegaly prolonged fever mediastinal tumors hemolytic anemia exfoliative dermatitis and spinal cord compression Infectious mononucleosis tuberculosis brucellosis sarcoidosis disseminated lupus erythematosus and carcinomatosis often present problems in differential diagnosis The difficulty can be resolved only by demonstrating the presence of one of the lymphoma group histologically or by satisfying the diagnostic criteria for the other disease under consideration

Stage II

In cases which are classified as Stage II surgical treatment is rarely employed and irradiation is usually the treatment of choice. Therapy is directed toward the involved lymph nodes, generally beginning in the most important area first. Irradiation is started cautiously in low daily dosage levels (about 100 r in air to one or two portals, the HVL depending on the site of the lesions). Therapy is aimed at local symptomatic relief and the amount of treatment is increased slowly. As little as 600 r tumor dose may produce

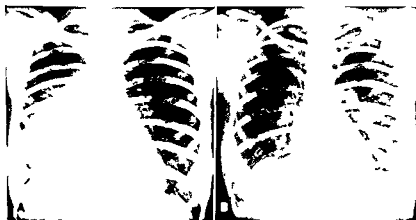


Figure 63 Chest roentgenograms of a patient with Hodgkin's disease. The diagnosis of the intrathoracic tumor (A) was established by biopsy. The patient responded unusually well to irradiation and had no evidence of recurrence twelve years later (B).

the desired result and the total seldom exceeds 3000 r.[9] Excessively rapid resolution of large masses and severe damage to the skin areas are avoided if possible. If constitutional symptoms such as fever, sweats, weight loss, and anemia are present, treatment with one of the alkylating agents is usually employed. If systemic symptoms are severe, chemotherapy may be given first and followed by irradiation. Chemotherapy may also be employed if the response to irradiation is poor.

Nitrogen mustard intravenously in a total dose of 0.4 mg. per kilo of body weight appears to be the drug of choice in Hodgkin's disease.[1, 30, 3, 60] It is often effective in the other diseases of the lymphoma group.

Chemotherapeutic agents that can be administered orally have obvious advantages for ambulatory patients. Chlorambucil, which is usually given in a dose of 0.1 to 0.2 mg. per kilo per day, is preferred to TEM because of its lower toxicity and greater predictability. It may be the alkylating agent of choice in the treatment of lymphosarcoma and giant follicle lymphoma.[13, 0] Good responses may not occur until after from six to ten weeks of treatment. TEM is a difficult drug to regulate because patients vary a great deal in responsiveness and the effect of a single tablet cannot be evaluated for two or three weeks.[31] The drug is administered until the desirable clinical result is

Clinical Classification of Malignant Lymphoma According to Extent of Disease

Stage I	Involvement of only one lymph node region or a single lesion elsewhere with no constitutional symptoms
Stage II	Involvement of two or more proximal lymph node regions confined to either upper or lower trunk with or without constitutional symptoms
Stage III	Involvement of multiple lymph node regions with or without constitutional symptoms or acute Hodgkin s disease with no obvious lymphatic involvement

Stage I

Patients with Hodgkin s disease lymphosarcoma reticulum cell sarcoma or giant follicle lymphoma which is classified as Stage I should be considered for surgical attempts at eradication of the disease The results of surgical treatment are best if the disease is confined to the stomach or the superficial nodes in the cervical region This approach is based on the concept that the disease in question originates in one focus Reports of the results obtained *with surgical treatment of all diseases of the lymphoma group*[18] *suggest* that a radical surgical approach may be justified in selected patients The results reported in 55 patients treated in this manner indicate a median *survival time of over five years and relatively frequent survival of eight to* ten years in all types of lymphoma The average survival time of these dis eases treated by other methods is probably two or three years The results of surgical treatment are difficult to evaluate because the surgically treated group is a selected one in which the disease appeared localized and includes no instances of extensive disease which might be expected to have a poor prognosis Nevertheless the frequency of long term survival in patients with lymphosarcoma of the gastrointestinal tract treated by surgical methods with or without irradiation indicates that in some patients at least the disease tends to remain localized for relatively long periods [9, 54] In such a situation *the disease is amenable to surgical treatment An attempt at surgical cure* appears to be a more logical approach for reticulum cell sarcoma lympho sarcoma and giant follicle lymphoma than for Hodgkin s disease but it has been recommended in selected cases of Hodgkin s disease[11]

Whether or not a surgical cure is attempted in a patient who is classified as Stage I an intensive course of irradiation is given Patients with Hodgkin s disease receive 3000 to 3500 r tumor dose in two or three weeks the half value layer depending on the location of the lesion Patients with lymphosarcoma and reticulum cell sarcoma are given 4000 to 5000 r tumor dose in four to six weeks if possible[9] Blocks of tissue that include the lymphatic *chain for a generous distance beyond the obvious involvement are irradiated* Whether or not chemotherapy should be administered routinely because of the possibility that the disease is present in other parts of the body is still undetermined It is not our policy to administer chemotherapy routinely

Patients with masses in the superior mediastinum, compression of the spinal cord, or involvement of the central nervous system are treated cautiously and kept under close supervision. Serious consequences may follow any increase in pressure in the lesion or adjacent tissues that occurs as a reaction to treatment. When a lesion of this type is irradiated only small dosage is employed. A steroid drug is often given in this situation in an effort to reduce or prevent the inflammatory reaction in the lesion that is sometimes produced by irradiation. The use of nitrogen mustard or TEM has been recommended in these situations because of the belief that the effects of these chemicals are different from those of x rays and induce regression of the lesion without producing active congestion or edema.[8] However, at least one patient with Hodgkin's disease who had signs and symptoms of spinal cord compression developed temporary paraplegia twice after courses of nitrogen mustard.[39] No form of treatment eliminates the need for caution and careful observation in patients of this type during the initial stages of therapy.

Prognosis

The survival time in patients with Hodgkin's disease who are treated varies from a few months to more than twenty years.[3] The outlook is poorest in Hodgkin's sarcoma where the average survival time has been found to be about one year.[16, 7] Even in this type of disease survivals of ten and twenty years have been reported.[15] The prognosis is better in the other types of Hodgkin's disease and the average survival time is from 2.5 to 3.2 years.[16, 9, 7]

Jackson and Parker recognized three different types of Hodgkin's disease histologically—paragranuloma (10 per cent), granuloma (78 per cent), and sarcoma (12 per cent). They reported a relationship between the prognosis and the histologic appearance of the lesion. The prognosis was best in patients with paragranuloma and poorest in those with sarcoma.[8] Some authors have agreed with this opinion, and others have considered this viewpoint true in general but not applicable to the individual patient.[15] When no distinction has been made between the histologic types of Hodgkin's disease the five year survival has been reported to be 10 per cent,[9] 17 per cent[36] and 23.6 per cent.[1] A ten year survival rate of 13.2 per cent has been reported.[1] The prognosis is less favorable in patients who have a generalized form of disease,[17, 18] fever,[38] leukocytosis,[38, 41] anemia,[46] and rapid recurrences after therapy.[16] The sex of the patient has little influence on the prognosis.[5] Although there is no definite correlation between age and survival, patients under 40 years of age appear to tolerate the disease better than older individuals.[1]

It is difficult to gain an accurate picture of the prognosis in lymphosarcoma and reticulum cell sarcoma because of the different histologic and clinical classifications that have been used. In the report of Minot and Isaacs the term "lymphoblastoma" was used to include Hodgkin's disease, lymphosarcoma and other forms of sarcoma involving the lymphatic system

obtained or undesirable depression of the bone marrow occurs According to one schedule 2 5 mg is administered daily for two or three days and the maintenance dose is varied according to the initial sensitivity as judged by the effect on the leukocyte count and appetite [9] In the majority of patients with lymphoma the subsequent schedule of 2 5 or 5 0 mg per week is satisfactory during the initial course of therapy However leukocyte counts are performed each week and the decision about treatment is withheld until the count is known If the leukocyte count falls to less than 3500 per cu mm it is advisable to defer further drug administration for at least three weeks in most circumstances

Stage III

Some patients whose disease is classified in Stage III are free of constitutional symptoms and may be managed by irradiation directed at the involved areas Most patients in this category sooner or later develop marked constitutional symptoms and receive chemotherapy one or more times The usual course of nitrogen mustard consists of four intravenous injections of 0 1 mg per kilo each [9] but a fifth dose from three to six days later has been recommended [11] In order to shorten the patient s hospital stay and reduce the number of gastrointestinal reactions some physicians administer the total amount of nitrogen mustard in a single intravenous injection of 0 4 mg per kilo [9] Courses of nitrogen mustard are not repeated in less than six or eight weeks unless urgently needed [3] In the lymphomatous diseases excellent responses often follow chemotherapy and sometimes the remission persists for a year or more Treatment of patients with advanced disease aims at palliation not cure and irradiation is employed mainly to treat lesions that are producing symptoms

The adrenal corticosteroids are useful in the treatment of selected patients Although the lymph nodes rarely show much decrease in size as a result of treatment with these compounds regression of splenomegaly tumor masses and hepatomegaly has been observed in both lymphosarcoma and Hodgkin s disease [47] More often the patient improves symptomatically In many patients there is an improvement in appetite associated with a diminution in fever and pain and in some there is an increase in hematopoiesis [47] These preparations are particularly useful in patients who develop thrombocytopenic purpura or hemolytic anemia When one of the steroid drugs is given in addition to more specific measures to patients with lymphoma who have auto immune hemolytic anemia an excellent response can be expected in about one half the patients in about 15 per cent the response is poor [38]

Splenectomy is sometimes performed on selected patients with these diseases when severe leukopenia, thrombocytopenia or hemolytic anemia occurs and fails to respond to other measures The procedure appears to be particularly effective in patients with giant follicle lymphoma [14] The response to splenectomy in patients with lymphoma who have auto immune hemolytic anemia is unpredictable· probably less than half of such patients respond to this procedure but in some the results are excellent [38]

interpreted as "hyperplastic lymph node" Two months later the patient developed night sweats pleural effusion and ascites A repeat biopsy of a cervical node at this time was interpreted as "Hodgkin s disease"

During the next 12 years the patient received therapy at intervals that varied from six months to three years She was usually treated with high voltage radiation but at various times was treated with nitrogen mustard TEM and chlorambucil On nine occasions courses of x ray therapy were given to the mediastinum spleen and various superficial nodes During all this time she was managed on an ambulatory basis and led an active life

In the spring of 1958 the patient developed anemia (hematocrit of 27 per cent) thrombocytopenia (platelet count 20 000 to 40 000 per cu mm) and leukopenia (white cells 1000 to 1500 per cu mm) It was thought that the pancytopenia was the result of splenomegaly associated with Hodgkin s disease or a combination of this factor and the effect of treatment on her bone marrow She was treated with prednisone frequent transfusions and small doses of chlorambucil and was only slightly incapacitated until October 1958 At this time she was admitted to the hospital with a history of severe frontal and occipital headache of eight days duration Six days prior to admission she noticed ecchymoses around the eyes and complained of diplopia She also reported transient weakness and numbness of the left hand and wrist The hematocrit was 21 per cent white count 1400 per cu mm and platelets 3000 per cu mm Marked weight loss was apparent It was thought that the patient had advanced Hodgkin s disease and that she had suffered an intracranial hemorrhage because of the thrombocytopenia When lumbar puncture was performed the opening pressure was found to be 600 mm The fluid contained 250 red cells and 80 neutrophils per cu mm The spinal fluid protein was 10 and the sugar was 48 Examination of the cerebrospinal fluid revealed numerous Torula organisms Despite treatment with prednisone amphotericin B and other measures the patient s condition deteriorated rapidly generalized convulsions developed and she died a few days later twelve years and two months after the onset of the first symptoms

Postmortem examination revealed splenomegaly and enlargement of the lymph nodes The presence of Hodgkin s disease was identified in the lymph nodes However the striking finding was the severe generalized systemic infestation with Torula organisms which were present throughout the lungs kidneys brain and meninges

Comment This patient is somewhat unusual in that she survived for over twelve years with Hodgkin s disease She responded well to different forms of treatment on numerous occasions even though the disease was generalized when first diagnosed She illustrates the fact that generalized disease at onset does not necessarily mean a poor prognosis As sometimes happens in diseases of the lymphoma group the initial node biopsy did not reveal the diagnosis The final episode was generalized torulosis with meningitis death appeared to be due to this infection rather than the progression of the Hodgkin s disease Torulosis which is not an uncommon development

The duration of disease in this series of 163 patients who were not treated with irradiation was 2 45 years[43] In lymphosarcoma the average survival time has been reported to be 2 3 years (lymphoblastic lymphoma" 1 4 years "lymphocytic lymphoma 3 3 years)[16] 1 5 years[illegible] and 2 3 years[43] The five year survival rate has been found to be 26 3 per cent[10] and 19 per cent In one series the ten year survival rate was 17 per cent[6] In a series of 182 patients with "lymphosarcoma (a diagnosis that included "lympho blastic "lymphocytic clasmatocytic" and "reticulum cell" sarcoma) the five year survival rate was 24 per cent[4] In the group of 95 patients with disease largely confined to the peripheral lymph nodes the mean duration of illness was 2 8 ± 0 3 years and the five year survival rate was 23 2 per cent In a group of 52 patients whose clinical manifestations were caused mainly by masses in the thorax or abdomen the mean duration was 1 4 ± 0 2 years and the five year survival was 1 9 per cent In a special group of 44 patients with clinical evidence of disease limited to one organ (gastrointesti nal tract oropharynx skin bone) the mean duration of illness was 3 4 years and the five year survival rate was 38 6 per cent[41] Average survival times of 1 8 years[4] and 1 9 years ("stem cell lymphoma and "clasmatocytic lym phoma")[16] have been reported in reticulum cell sarcoma

It is generally agreed that giant follicle lymphoma has a better prognosis than the other diseases in the lymphoma group Average survival times of 3 5 years 5 6 years[10] and 6 0 years[1] and a mean duration of 4 4 ± 0 5 years[1] have been reported Five year survival rates of 50 per cent[17] and 45 8 per cent[41] and ten year survival rates of 11 per cent[1] and 8 3 per cent[41] have been reported

It is virtually impossible to predict accurately the survival time of an individual patient In general the prognosis is best when the disease is apparently limited to a group of superficial lymph nodes and is unaccom panied by constitutional symptoms such as fever sweating fatigue weight loss changes in the peripheral blood or splenomegaly Although exceptions occur patients with generalized disease especially those with constitutional symptoms and marked changes in the peripheral blood have a worse prog nosis Patients with lymphosarcoma confined to the stomach who are treated surgically with or without irradiation have a better than average prog nosis[1] In the other types of lymphoma it has not been shown that therapy prolongs life significantly[10, 43, 1] although it appears to be life saving in certain patients

ILLUSTRATIVE CASES

Hodgkin's Disease

This patient was 21 years old in 1946 when it was noted that her abdomen did not return to normal size following delivery of a normal child One month later she was found to have enlargement of the inguinal nodes cervical nodes and right axillary nodes Biopsy of a lymph node at this time was

interpreted as "hyperplastic lymph node" Two months later the patient developed night sweats pleural effusion and ascites A repeat biopsy of a cervical node at this time was interpreted as "Hodgkin s disease"

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in patients with lymphoma was apparently present in this patient for some months without being recognized

Reticulum Cell Sarcoma

This 53 year old white male realtor was admitted to the hospital three weeks after an attack of "flu" that was characterized by malaise and mild fever Two weeks prior to admission he developed progressive dyspnea He was found to have a right hydrothorax and thoracentesis was performed on several occasions with removal of 1 to 1 5 liters of fluid Because the fluid reaccumulated rapidly he was referred to the hospital for study

Physical examination revealed the signs of hydrothorax on the right In addition the right axillary nodes measured 2 to 3 cm and the left axillary nodes measured 1 to 2 cm Examination of the cell blocks prepared from pleural fluid was inconclusive on several occasions Biopsy of an axillary lymph node revealed lymphosarcoma The remainder of the physical and laboratory examinations were normal

This patient received a course of x ray therapy totalling 1800 r to each side of the chest in August 1949 In addition he received 0 4 mg per kilo gram of nitrogen mustard intravenously He responded well symptomatically but irradiation was necessary in August 1950 in October 1950 and again in September 1951 Nitrogen mustard was administered in August 1952 and in September 1952 In the fall of 1952 cortisone was administered

At the end of January 1953 the cervical and submental lymph nodes enlarged rapidly the appetite failed and the patient lost weight and was readmitted to the hospital Examination disclosed generalized enlargement of the superficial lymph nodes right and left hydrothorax hepatomegaly splenomegaly and numerous other intra abdominal masses The hemoglobin was 6 gm per 100 ml the red count was 2 0 per cu mm the white count was 2800 per cu mm and the differential count was normal The blood urea nitrogen was 100 mg per 100 ml Other laboratory studies were essentially normal Despite treatment with transfusions and other measures the patient's condition deteriorated steadily and he died three weeks after admission three and one half years after the onset of his symptoms It was thought that the terminal episode was pneumonia

Postmortem examination revealed large retroperitoneal tumors which were obstructing the ureters There was generalized enlargement of the lymph nodes liver and spleen Areas of tumor were present in the kidneys ribs lungs and vertebrae Areas of pneumonia were present in both lungs The bone marrow was partly fatty but areas of hyperplasia were present The final diagnosis at autopsy was reticulum cell sarcoma

Comment This case illustrates many of the usual features of the lymphoma The disease first appeared in middle age Several regions were involved when the patient first consulted his physician Histologic study of the pleural fluid did not establish a diagnosis but a diagnosis of lymphosarcoma was made on a biopsy of an axillary lymph node The patient's disease responded well to irradiation and nitrogen mustard for several years

However in the last few months of his life the tumor progressed rapidly and his illness terminated with an episode of pneumonia. The initial biopsy was interpreted as lymphosarcoma and at autopsy a diagnosis of "reticulum cell sarcoma" was made Distinction between the two is often difficult and at times the biopsy diagnosis differs from the autopsy diagnosis even when the sections are reviewed by the same pathologist as was true in this patient Fortunately such differences in diagnosis usually have little effect on the management of the patient.

Giant Follicular Lymphoblastoma

This 54 year old white housewife consulted her physician in November 1957 because of an enlarged lymph node in the left side of the neck. The patient reported that six months earlier she had noticed the transient enlargement of a node in the left supraclavicular area at the time of an upper respiratory infection Swelling of the node recurred several months later during an attack of "bronchitis The enlargement again subsided but reappeared a month prior to examination and persisted during the interval Physical examination revealed moderately firm nodes that measured 1 to 2 cm. in the left submandibular area left supraclavicular area left axilla and right inguinal area The remainder of the physical examination was normal Urinalysis hematocrit, leukocyte count and differential leukocyte count were normal A left supraclavicular node was excised for study and was reported to be "giant follicular lymphoblastoma."

The patient received no treatment but felt well until November 1958 when she had an episode characterized by fever malaise and abdominal discomfort. The fever subsided and she improved somewhat but continued to have abdominal distention mild right lower quadrant pain, and irregular attacks of mild diarrhea. During this time she noted progressive weakness but lost no weight. The physical examination appeared unchanged but the leukocyte count increased gradually until March, 1959 when it was 22 600 per cu.mm At the same time the hematocrit was 37 per cent. Roentgenographic studies of the chest, stomach small bowel and colon were all considered to be normal Because of her continued symptoms she was referred to the University Hospital

At the time of admission a moderately firm slightly tender ill defined mass was palpable in the right lower quadrant Moderately firm lymph nodes that measured 2 cm in diameter were felt beneath the left mandible in the left supraclavicular area, and in both axillary and inguinal regions The liver and spleen were not palpable The remainder of the examination was noncontributory

Laboratory examinations hematocrit 37 per cent white count 22,000 per cu mm differential count (per cent) bands 6 segmented 76 lymphocytes 16 monocytes 1 eosinophils 1 The platelets were 374 000 per cu mm. Urinalysis was normal Bone marrow examination revealed marked generalized hyperplasia.

Laparotomy was performed because it was thought that the mass in the

right lower quadrant was a tumor of lymphomatous origin probably Hodgkin s disease At operation it was found that the mass that had been palpated consisted entirely of omentum Both the colon and small intestine were free of obstruction However greatly enlarged lymph nodes were found in the root of the mesentery and in the periaortic area Histologic study of the omental mass revealed chronic inflammation and a lymph node biopsy was interpreted as Hodgkin s disease

The patient s postoperative course was uneventful She was treated with irradiation to the abdominal areas and two months later was given a course of nitrogen mustard intravenously 0 4 mg per kilo of body weight

Comment This patient s course illustrates the transformation of the histologic picture of giant follicular lymphoblastoma or Brill Symmers" disease into that of Hodgkin s disease Although some patients with giant follicular lymphoblastoma follow a relatively benign course for many years not infrequently the clinical manifestations of the disease change when the histologic picture alters to that of Hodgkin s disease lymphosarcoma or lymphocytic leukemia When the diagnosis of giant follicular lymphoblastoma is made the patient is usually treated with irradiation or chemotherapy as is customary with the other diseases in this group When the diagnosis of Hodgkin s disease was made in this patient she was treated with irradiation to the abdominal region because of the major involvement of this area and with an alkylating agent because of the widespread nature of the disease

Lymphosarcoma

This 65 year old white saleslady was well until December 1955 when she noticed small purpuric lesions on the palms of the hands During the next month the cutaneous lesions increased and became generalized These lesions persisted and during the following six weeks the patient noticed cramping pains and edema in the legs mild anorexia and weight loss of 10 pounds She was admitted to the hospital on March 17 1956

Physical Examination Signs of hydrothorax were present bilaterally The spleen was palpable three fingerbreadths below the left costal margin and multiple orange sized masses were palpable throughout the abdomen Pitting edema was present in both legs The trunk back and upper arms were covered with maculopapular hemorrhagic rash The remainder of the examination was essentially normal

Laboratory Examination Hematocrit white count differential count urinalysis blood urea, stool examination and prothrombin times were normal Roentgenogram of the chest revealed small bilateral pleural effusions Bone marrow examination disclosed the normoblastic type of erythropoiesis associated with a moderate increase in lymphocytes a pattern that was thought to be consistent with lymphosarcoma or lymphocytic leukemia Biopsy of an axillary lymph node was diagnosed as lymphosarcoma "

Course She was treated with a total of 12 5 mg of TEM in three doses and was discharged from the hospital She was readmitted three weeks after

the last dose of TEM and four weeks after the first dose with a four day history of fever unproductive cough pain in the left chest and dyspnea The intra abdominal masses had become much smaller and the skin lesions had cleared almost completely On admission the hematocrit was 35 per cent, white count 250 per cu mm and the platelet count 136 000 per cu mm Thoracentesis was performed on several occasions and penicillin was ad ministered because of the granulocytopenia and pneumonia. She improved promptly and ten days after admission the white count had risen to 2200 per cu mm One month later two months after the last dose of TEM the hematocrit was 40 per cent the white count 4000 per cu mm. and the differ ential count was normal

The patient was largely asymptomatic and received no treatment for a year when enlargement of the supraclavicular lymph nodes and intra abdominal nodes recurred The patient was given 5 mg of TEM and de veloped nausea, vomiting and diarrhea despite the fact that she had taken chlorpromazine before the TEM The patient s abdominal nodes decreased in size remarkably The patient felt well for five months at which time the superficial and intra abdominal lymph nodes again increased in size At this time a prolonged course of TEM consisting of a total of 57 5 mg was ad ministered over a six month period At the end of this time the axillary and supraclavicular nodes measured about 2 cm in diameter the intra abdominal masses remained but the patient was largely asymptomatic and continued to work.

Five months after her last dose of TEM (eighteen months after her first admission) swelling of the legs and marked enlargement of the superficial and intra abdominal nodes recurred Treatment with chlorambucil 6 mg a day was started when the leukocyte count was 3000 per cu mm One month later there was less swelling in the abdomen the superficial nodes were no longer enlarged the intra abdominal masses appeared smaller and the dose of chlorambucil was reduced to 4 mg per day Two months after beginning chlorambucil no enlargement of the superficial lymph nodes could be de tected and the intra abdominal masses were just palpable Four and a half months after chlorambucil was started the patient reported that she was entirely asymptomatic Examination at this time revealed no enlargement of the superficial lymph nodes liver spleen or any detectable intra abdomi nal masses This excellent remission lasted for six months without further treatment

Comment The course of the lymphosarcoma in this patient illustrates many instructive features (1) the onset with skin lesions and gene alized involvement of the lymph nodes (2) the decision to treat the patient with an alkylating agent rather than irradiation because of the widespread dis tribution of the disease (3) the occurrence of severe granulocytopenia three to four weeks after the administration of TEM (4) the excellent re missions obtained with small amounts of TEM early in the course of the disease (5) the variable incidence of toxic manifestations after administra tion of different amounts of this drug (6) the increasing resistance of the

lymphosarcoma to TEM (7) the excellent remission obtained with chlorambucil after the patient had become resistant to TEM (8) the time required to obtain the remission with chlorambucil (four and one half months) (9) the relatively good prognosis of some patients with lymphosarcoma that is already widespread when the patient is first seen

MULTIPLE MYELOMA

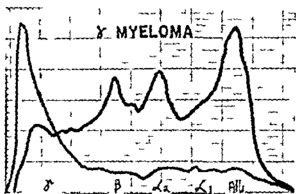
Multiple myeloma is an unusual and interesting disease in many respects The symptomatology is extremely varied and striking abnormalities are often apparent on the physical and roentgenologic examinations The introduction of the needle aspiration of the bone marrow has made the tumor cells easily available for histologic study and a number of different cellular abnormalities have been observed The disease has been of wide interest to investigators because it is characterized by a number of metabolic disturbances particularly in protein metabolism Although multiple myeloma is a rare disease it is important because a better understanding of the abnormalities in protein metabolism that occur in this disease will probably contribute to a better understanding of normal protein metabolism and antibody formation

HISTORICAL

Multiple myeloma was first reported in 1847 in a tradesman who presented with "mollities ossium (soft bones) and urine containing large quantities of animal matter The patient was studied by Drs MacIntyre and Watson and a urine specimen was sent to Dr Henry Bence Jones who described the peculiar characteristics of the unusual protein found in the urine[6] *The protein which he described is still known as* Bence Jones protein The name multiple myeloma was given to the disorder by von Rustizky in 1873[64] Prior to 1900 it was thought that different cellular types were responsible for the tumor in different individuals In 1900 Dr James H Wright established that the myeloma cell is related to the plasma cell and concluded that the neoplasm arises from an abnormal proliferation of these cells[9] These conclusions have been confirmed many times particularly since the introduction of marrow aspiration by Arinkin in 1929[6]

PATHOGENESIS AND PATHOLOGIC PHYSIOLOGY

Multiple myeloma is considered to be a malignant neoplasm that arises in the bone marrow and involves the skeleton primarily The clinical features



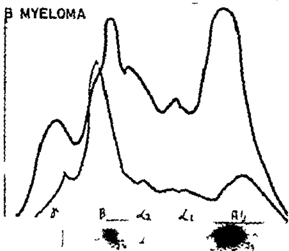


Fig. c 64 Paper electrophore is of serum and urine in multiple myeloma. In A the abnormal protein is found in the urine but is not apparent in serum. B The abnormal prot n i found in both the urine and the serum

of the disease are attributable to the location of the tumor and to abnormalities in protein metabolism While multiple myeloma appears to be primarily a widespread disorder of the bone marrow solitary osseous tumors and extraskeletal nests of myeloma cells are found occasionally Whether these lesions are metastatic from some skeletal site or arise from precursors in situ in response to some stimulus is unknown[80] A tumor which has many of the characteristics of multiple myeloma has been established in a strain of mice Studies of the behavior and metabolic characteristics of this tumor will probably give additional information about multiple myeloma in man[84] Multiple myeloma has also been studied in tissue culture[85] These studies provide evidence that both Bence Jones protein and gamma globulin are produced by myeloma cells grown in vitro

Myeloma cells are apparently responsible for the production of the abnormal proteins that are found in the serum and urine of patients with the disease Recent evidence indicates that the abnormal urinary and serum proteins that occur in any one case of multiple myeloma are separate and distinct from all other myeloma proteins as well as from the normal serum proteins[86-88] The most common protein abnormalities are the occurrences of Bence Jones protein a marked increase in the gamma globulin and the occasional presence of cryoglobulin Less often a form of primary amyloidosis is seen

The Bence Jones protein in the urine is not a derivative of the abnormal *serum proteins but is formed de novo in the body from amino acid precursors* at a rate that exceeds the rate of production of the abnormal serum proteins As much as 20 or 30 gm of Bence Jones protein may be excreted in the urine each day When it is recalled that from 70 to 90 gm of protein is available in the daily diet of the adult it is apparent that the amount excreted as Bence Jones protein constitutes a considerable diversion of protein from the usual metabolic needs This remarkable excretion of protein continues independently of any exogenous source of protein[8] The prolonged excretion of Bence Jones protein in the urine may produce renal insufficiency Apparently the protein is not specifically toxic to the renal tissue but is precipitated in the tubular lumen and in this way it leads to the eventual loss of the proximal nephron unit[3] Vacuolation and precipitation of protein have been observed within the tubular epithelium[89] and it has been suggested that these tubular lesions may be the cause of renal tubular reabsorption defects like those of the adult Fanconi syndrome For some obscure reason nitrogen retention which results from renal lesions in multiple myeloma is rarely accompanied by the hypertension usually seen in other forms of renal disease severe enough to produce azotemia

As a result of the increased production of protein the level of the serum protein particularly the globulin fraction is usually increased in multiple myeloma The increase usually involves the gamma globulin but in some instances the alpha and beta fractions are most affected Despite the large amount of gamma globulin that is often present in the circulating blood these patients are usually able to respond with only a poor antibody response to

antigenic challenge [68] It is not known whether this deficiency is the result of the diversion of protein needed for antibody formation into other channels or of a disorganization of antibody formation in the plasma cells The increased susceptibility of patients with multiple myeloma to pulmonary infection may be a consequence of the increase in serum protein which causes an increase in the blood viscosity and leads to thrombosis of the small pulmonary vessels Pulmonary infection is also favored by the subnormal antibody response and the decreased pulmonary ventilation that is secondary to painful fractures of the ribs or vertebrae [69]

Another example of the profound alteration in protein metabolism in this disease is the occasional occurrence in the serum of a cold precipitable globulin This protein known as cryoglobulin is found most often in multiple myeloma but may also occur in other diseases When it is present in large quantities the cryoglobulin may precipitate in the blood in areas of lower temperature such as the ears nose fingers or toes and in this way produce thrombosis ulceration or gangrene Raynaud's phenomenon may occur in these circumstances [71] As the amount of cryoglobulin increases the critical temperature rises with the result that precipitation sometimes occurs in deeper structures in the body

An unusual abnormality of protein metabolism a form of amyloidosis is seen in approximately 5 or 10 per cent of the patients with multiple myeloma [6, 60, 69] The distribution of amyloid material is similar to that seen in primary amyloidosis It has been found in the bones voluntary muscles skin liver spleen gastrointestinal tract adrenal glands kidneys tongue heart and synovial tissues In the latter circumstance clinical features that resemble rheumatoid arthritis may occur

Hypercalcemia is not unusual in multiple myeloma [64, 6, 8, 60, 69] It may occur as the result of hyperproteinemia which affords the plasma a greater calcium binding capacity or as the result of bone destruction with a release of more calcium into the blood than can be excreted by the kidneys The decreased renal function often found in multiple myeloma may also contribute to the hypercalcemia [1] Serum calcium levels as high as 16 or 18 mg per 100 ml occur without any significant disturbance in the levels of the serum phosphorus or alkaline phosphatase Gastrointestinal dysfunction weakness apathy polydipsia and polyuria are found in association with moderate increases of serum calcium while very high levels of serum calcium may lead to vascular collapse and death [61]

The anemia in multiple myeloma is due mainly if not entirely to decreased erythropoiesis It is interesting that a disease characterized by such marked disturbance of protein metabolism is so rarely associated with the auto immune type of hemolytic anemia

CLINICAL MANIFESTATIONS

Multiple myeloma occurs in all races and is about twice as common in males as in females [69] Prior to the introduction of sternal biopsy it was re

ported to constitute 0.03 per cent of all malignancies.[4] It is characteristically a disease of older persons. The disease has been reported in patients less than 20 years of age[66] but 95 per cent of the patients in one series were over the age of 40 years.[69] In a group of 50 patients reviewed by the authors all were past 40 years of age and 80 per cent were over 50 years of age.[69]

The commonest presenting symptom is pain, which occurs some time during the course of the disease in nearly all patients. Unfortunately it is often disabling and difficult to control. The pain is due to involvement of the bones and to pressure on the adjacent nerves. Compression fractures of vertebrae, which occur in about one half of the patients, are most likely to be found in the upper lumbar and lower dorsal vertebrae.[64, 4, 69] Pathologic fractures of the ribs are common and fractures of the femur, sternum, ilium, humerus, clavicle, and pubic bones are not rare. In some patients the discomfort is mild and poorly localized but in others the occurrence of a fracture is accompanied by the sudden onset of severe girdle-like pain in the chest or abdomen. Pain with and without fracture occurs commonly in the hips, legs, shoulders, and arms, but is rare in the neck and head. At times pain is not a prominent feature and the history is mainly that of recurring attacks of fever or pneumonia. The diagnosis of such episodes may be puzzling if other manifestations of multiple myeloma are not evident. Attacks of this nature, which were seen in 50 per cent of one series, are most likely to occur in patients with greatly elevated serum globulin levels.[4, 69] Whether these transient pulmonary manifestations are episodes of pulmonary infarction or pneumonia is uncertain. The clinical picture is usually that of fever, cough, mucopurulent sputum, and roentgenologic evidence of patchy infiltration in the lungs.

The frequent occurrence of radicular pain and evidence of cord compression in multiple myeloma is well known but the occurrence of peripheral neuritis without compression is not so widely recognized. The frequency of all types of neurologic involvement in multiple myeloma has been placed at 10 per cent,[6] 14 per cent,[64] 18 per cent,[8] and 40 per cent.[4, 69] In the last series cord compression, with or without paraplegia, was seen in about 14 per cent of the patients, all of whom had roentgenologic evidence of compression fracture of the spine. Thirty per cent of those with neurologic manifestations evidenced involvement of the spinal roots or peripheral nerves in the absence of vertebral collapse. Involvement of a nerve was manifested by radicular pain or other evidence of peripheral neuritis such as foot drop, hypesthesia, paraesthesia, weakness, or muscular atrophy. Cranial nerve involvement appears to be unusual.

Renal insufficiency occurs not infrequently in multiple myeloma and may dominate the clinical picture.[64, 6, 8, 69] Renal failure, which is secondary to involvement of the kidney by the disease, is unusual in the absence of Bence Jones proteinuria.[69] Hypertension is usually absent in these cases even when severe azotemia is present. The occurrence of azotemia without hypertension should arouse the suspicion of multiple myeloma.

Other less common manifestations of multiple myeloma are psychotic episodes often associated with marked elevation of the serum calcium, arthri

tis associated with deposits of amyloid in synovial membranes and the presence of palpable tumors overlying the involved bones

Significant loss of weight during the course of the disease was observed in 63 per cent of one series [8]

Physical examination may disclose nothing more than is usually found in patients of the same age group Probably the most frequent finding is tenderness on pressure over the areas of osseous involvement Palpation should be gentle in these patients One of the authors once had the experience of producing a fracture of the sternum while testing for sternal tenderness and then produced another in the humerus when he helped the patient change position Among the commonest signs on physical examination are those associated with compression of the spinal cord and involvement of the peripheral nerves Palpable tumor masses are seen in a minority of patients and then usually only in the later stages of the disease [64 69] Moderate enlargement of the superficial lymph nodes is seen frequently Palpable enlargement of the liver occurs in from 11 to 40 per cent of the patients and in from 2 to 25 per cent the spleen is moderately enlarged [69]

Abnormalities of the skeleton that are demonstrable by x ray constitute one of the most characteristic features of multiple myeloma The most typical changes are the presence of multiple clearly defined punched out lesions



Figure 65 Roentgenogram of a patient with multiple myeloma showing the characteristic "punched out" lesions

that involve several different bones (Fig 65) The size of the lesions varies from a diameter of 1 or 2 mm to areas as large as 10 to 12 cm the lesions are *characterized by the absence of any osteoblastic reaction* [83] In some patients such definite lesions are absent and roentgenograms show only generalized demineralization similar to that seen in senile or postmenopausal osteoporosis In both types of lesions pathologic fractures particularly *compression fractures of the vertebral bodies* are *common* *Since the* advent of sternal puncture and more detailed study of the plasma proteins as diagnostic measures it has become evident that some patients perhaps 5 to 12 per cent with undoubted myeloma have no demonstrable abnormalities *in the roentgenograms at the time the diagnosis is made* [64,7,80,89,90] Demonstrable lesions occur most commonly in the spine ribs sternum and clavicle lesions of the skull extremities pelvic girdle and shoulder girdle occur about half as often [4,83] Lesions of the mandible occur less often

LABORATORY EXAMINATION

Many abnormalities occur in the peripheral blood in patients with multiple myeloma Anemia is one of the commonest features and is rarely absent throughout the course of the disease In one series the hemoglobin ranged from 6 to 10 gm per 100 ml and the erythrocyte count ranged from 2 to 4 mil per cu mm [89] The anemia is usually normocytic but when severe it may be macrocytic The leukocyte count is usually normal but in one large series 20 per cent of the patients had leukocytosis and 40 per cent had leukopenia [89] Examination of a smear of the peripheral blood may suggest the diagnosis because of the presence of rouleau formation or plasma cells or other abnormal mononuclear cells that resemble the abnormal cells found in the bone marrow These cells may become so numerous in the peripheral blood that a diagnosis of "plasma cell leukemia" is made Normoblasts and myelocytes appear in the peripheral blood not infrequently but are most likely to be seen in the advanced stage of the disease The platelet count is normal in most patients but thrombocytopenia has been reported to occur in about 25 per cent [89]

Examination of bone marrow obtained by aspiration reveals a diagnostic or suspicious cell pattern in approximately 80 per cent of the cases of multiple myeloma [9] *The most characteristic finding is the presence of "myeloma" cells* which may constitute from 4 to 98 per cent of the cells present [67,9] These cells which vary from 15 to 40 microns in diameter usually have a single eccentrically placed nucleus The chromatin pattern of the nucleus is finely divided and often contains several small nucleoli or a single large nucleolus Various types of inclusions are sometimes seen in the cytoplasm of myeloma cells Some cells appear vacuolated (Mott cell) and others contain protein particles that are thought to originate in the cell One such protein forms the Russell bodies which appear eosinophilic with Wright's stain and sometimes resemble whole erythrocytes or fragments of erythrocytes Other cells contain protein particles and have a bluish color when stained with Wright's stain and still others contain definite protein



Figure 66 A and B Plasma cells containing Mott bodies bone marrow aspiration multiple myeloma.

crystals In many patients the typical myeloma" cells are not seen but instead there is a large increase in cells that appear to be mature plasma cells It is extremely rare for such cells to constitute more than 30 or 40 per cent of the total cells in the marrow in any disease other than multiple myeloma Less pronounced increases in the marrow plasma cells occur in a number of disorders such as hypersensitivity states collagen diseases disseminated carcinomatosis chronic infection (particularly the granulomatous types) and cirrhosis of the liver[68 6 77 86 91] Multinucleated plasma cells are seen commonly both in myeloma and in other disorders

A serum protein abnormality that is demonstrable by electrophoresis

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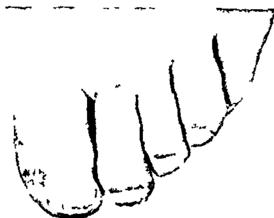


Figure 67 Purpuric lesions on toes of patient with multiple myeloma and cryoglobulinemia

occurs in about 75 per cent of patients with myeloma[83] An additional 18 per cent of patients who fail to show characteristic changes on serum electrophoresis have Bence Jones protein in the urine If electrophoresis of urine protein is performed in addition to the serum electrophoresis diagnostic abnormalities are found in over 93 per cent of patients with multiple myeloma[84] In the majority of patients the abnormality consists of a sharp peak in the gamma fraction of the serum protein but occasionally the increase is found only in the alpha or beta fraction[83] While a pattern of this type indicates the presence of a protein abnormality that is most likely associated with multiple myeloma, such changes are not diagnostic of multiple myeloma because they have been described in patients with lymphoma leukemia and amyloid disease Myeloma cannot be excluded by the occurrence of a normal electrophoretic pattern because on rare occasions a patient with multiple myeloma may have a normal serum protein pattern[67, 78, 83, 8] The total protein is found to be over 8 0 gm per 100 ml in about 72 per cent of patients[64, 89] The increase occurs in the globulin fraction which may amount to 10 or 11 gm per 100 ml Cryoglobulinemia the presence in the circulating blood of a globulin that precipitates at temperatures lower than 37° C occurs in only a small percentage of patients with multiple myeloma. Multiple myeloma is probably responsible for most instances of cryoglobulinemia particularly if the globulin is present in large amounts but such globulins are not diagnostic of multiple myeloma for they occur in other diseases particularly those of the lymphoma group as well

The protein originally described by Bence Jones is found in the urine of 40 or 50 per cent of the patients with multiple myeloma when the urine is examined in the usual fashion by differential heating and acidification When precipitin tests and electrophoresis are employed the abnormal protein can be demonstrated in virtually every case[0, 83]

Other abnormalities are often found in the peripheral blood The com

monest of these is a greatly elevated sedimentation rate which occurs in the vast majority of patients with multiple myeloma, particularly if the serum protein is increased. When rouleau formation is prominent in the blood smear the sedimentation rate is usually greatly increased. Hypercalcemia with values of 12 to 18 mg. per 100 ml. has been reported in from 20 to 39 per cent of patients.[44-46] Even when the calcium is markedly elevated the levels of the serum phosphorus and the alkaline phosphatase are normal or slightly elevated. As is usually true in patients with any type of chronic renal disease marked elevation of the urea nitrogen and other changes occur when renal failure develops in patients with multiple myeloma.

DIAGNOSIS

The classic case of multiple myeloma is usually recognized without difficulty. In a middle aged or elderly individual the occurrence of pain, particularly radicular pain associated with compression fractures of the spine and anemia suggests the diagnosis. If an increase in the level of serum globulin and Bence Jones proteinuria are demonstrated multiple myeloma is very probable. The diagnosis is established by finding "myeloma" cells or greatly increased numbers of plasma cells in the bone marrow; the presence of a sharp gamma peak on plasma electrophoresis usually has the same significance.

When many of the commoner features of the disease are absent or when some particular manifestation dwarfs the other features the diagnosis may be more difficult. In older persons multiple myeloma must be considered in the differential diagnosis of any unexplained anemia. The diagnosis also should be considered in patients whose roentgenograms show osteoporosis or lytic lesions of the skeleton; the absence of osteoblastic changes, involvement of multiple vertebral bodies, involvement of the skull, and particularly the mandible are all points that favor the diagnosis of multiple myeloma. Some patients with multiple myeloma come under observation first because of renal insufficiency, uremia, anemia, and proteinuria, and for this reason the diagnosis should be considered in all patients with these manifestations who are middle aged or older, especially if hypertension is absent. Not infrequently the patient consults a physician because of radicular pain, paraplegia, or some other neurologic difficulty and attention may first be focused on the nervous system. Perhaps the diagnosis of multiple myeloma is most likely to be missed in patients who present with fever and roentgenologic evidence of pneumonia and in those whose complaints are particularly suggestive of peripheral neuritis or arthritis. In such patients difficulties usually arise because of the failure to consider multiple myeloma in the differential diagnosis. If multiple myeloma is present the diagnosis can nearly always be established by careful study of the bone marrow and the electrophoretic pattern of the protein in the serum and urine. All three of these examinations are usually abnormal if multiple myeloma is present; failure to find evidence of multiple myeloma in any of the three practically excludes the disease from further consideration.

COURSE AND PROGNOSIS

Multiple myeloma is a fatal disease with an average survival time of less than two years from the onset of symptoms[64, 67, 4, 8, 81, 89] The median survival time in one series was 13 months[78] and in another 15 months[64] Although some patients succumb to the disease within a few weeks after onset of symptoms others occasionally survive for eight, eleven or more years[67, 0, 8] The three year survival rate appears to be only from 5 to 15 per cent[6, 70, 78] The prognosis is better if the disease appears to be localized[64, 6] in one group of 18 such patients 10 survived for more than two years and 3 survived from 3 8 to 9 8 years[67] The status of the solitary myeloma is uncertain At best it seems to be exceedingly rare because prolonged observation of patients suspected of belonging in this category almost invariably reveals widespread disease sooner or later sometimes after six to ten years have elapsed[64, 67, 74, 8, 80, 89] The prognosis in an individual case bears little relationship to the apparent degree of maturity of the tumor cells in the marrow[79, 89] Methods of treatment so far available have had little influence on the survival rate in the disease[64, 78] but undoubtedly there are individual exceptions[67]

A small percentage of patients with multiple myeloma lead active lives relatively free from symptoms for a period of years Unfortunately the vast majority have a very distressing course characterized by pain fractures disability progressive weakness and cachexia Remissions sometimes occur but these are usually short and the disease soon resumes its relentless painful and crippling course

TREATMENT

The treatment of multiple myeloma is unsatisfactory at best Non specific measures such as immobilization of fractures body braces for vertebral involvement, and analgesics for pain add greatly to the comfort of the patient If anemia is marked blood transfusions may be necessary because the anemia rarely responds to other measures Laminectomy is often necessary in patients with paraplegia and other surgical procedures may be warranted if the disease appears to be localized

The actual disease process responds poorly to more specific therapy Nitrogen mustard TEM radioactive phosphorus and stilbamidine have all proved ineffective[6, 9] Urethan is probably the most widely used form of treatment at the present time It is administered in a daily dose of from 2 to 5 gm for a period of six to ten weeks (total dose 90 to 300 gm)[87] or in a large initial dose of 6 gm a day for three to six days following which a maintenance dose of 3 gm daily is kept up indefinitely[78] Symptomatic improvement generally of relatively short duration probably occurs in from 20 per cent[8] to 30 per cent[79] of the patients so treated In a smaller number of patients objective evidences of improvement such as improvement in the anemia decrease in the serum globulin and improvement in the radiologic

appearance of the bone lesions have occurred[4, 5] No prolongation of the survival time of patients with multiple myeloma treated with urethan has been reported[4, 5] Disadvantages of this form of treatment are the frequent occurrence of nausea and vomiting and the occasional development of severe leukopenia or hepatic damage[5] If prednisone or cortisone is administered along with urethan the symptomatic improvement seems to be enhanced. When given alone adrenocortical steroids usually produce but slight symptomatic improvement, but occasionally there is relief of bone pain and a decrease in the abnormal globulin component in the serum and urine[61]

Roentgen irradiation has an important place in the treatments of some patients with multiple myeloma. When therapy is directed toward specific lesions pain is often relieved promptly and spontaneous fractures may heal more rapidly It has also been observed in some patients with relatively localized disease that therapy directed toward specific lesions may eradicate the evidence of disease in those locations[5]

Should hypercalcemia occur a high fluid intake should be administered to provide urinary excretion of calcium and by lowering urinary calcium concentration prevent nephrocalcinosis[63] The diet should be low in calcium Adrenocortical steroids have proved helpful in reducing the high calcium levels though their mechanism of action is unknown[61] Sodium ethylene diamine tetraacetic acid (EDTA) a chelating agent, has been used unsuccessfully in the treatment of hypercalcemia The results are transient and it may produce serious nephrotoxic effects and further renal impairment[63, 6]

ILLUSTRATIVE CASES

Case 1

A 55 year old white male barber enjoyed excellent health until six weeks before admission to the hospital when he first noticed a nagging non radiating pain in the lower portion of the back The pain was aggravated by bending, coughing and sneezing Two weeks prior to admission the pain suddenly became much more severe and radiated into the right leg The patient had noticed no weakness numbness or paresthesias in the legs

Physical examination disclosed pain on motion of the spine The liver was palpable 2 cm below the right costal margin The right knee jerk and right ankle jerk were diminished and there was a slight diminution of sensation to pin prick over the right calf and foot The physical examination was otherwise normal

Laboratory Examination Admission blood studies hct. 39 per cent, wbc 7600 per cu mm Urinalysis was negative except for a weakly positive test for Bence Jones protein The differential leukocyte count and blood urea were normal. The sedimentation rate was 23 mm at the end of one hour (normal 8 to 10) Serum calcium was 10.5 mg per 100 ml alkaline phosphatase 4 1 Total protein was 8 8 gm per 100 ml and plasma electro-

phoresis showed the following composition albumin 35% alpha 1 globulin 6 2% alpha 2 globulin 12 3% beta globulin 6 9%, beta prime globulin 6 3% and gamma globulin 31 9% The gamma fraction was greatly increased and formed a sharp peak an abnormality which was considered characteristic of myeloma Bone marrow examination revealed that about 67 per cent of the cells were myeloma cells" Many had finely divided nuclear chromatin and contained one or more nucleoli Roentgenogram of the spine revealed a destructive lesion in the anterior half of the fourth lumbar vertebra which was associated with some wedging

Course A diagnosis of multiple myeloma was made Roentgen ray therapy 4000 r in air was administered through two portals to the spinal lesion In addition the patient was given 3 gm of urethan a day This drug was discontinued at the end of two weeks because of nausea and vomiting Irradiation afforded moderate symptomatic relief initially but the pain soon recurred and the patient required opiates for relief His condition continued to deteriorate and he died six months after his initial admission eight months after the initial symptom of back pain

Comment This patient illustrates many features that occur commonly in multiple myeloma The onset was in the sixth decade and the initial symptom was back pain The roentgenogram revealed an osteolytic lesion in a lumbar vertebra the commonest site for this type of lesion The occurrence of Bence Jones protein in the urine the marked increase in gamma globulin in the serum protein and the presence of "myeloma cells" in the bone marrow confirmed the diagnosis The radiation of the pain and the alterations in the tendon reflexes and sensory perception indicated involvement of the nerve roots As so often happens the patient responded poorly to treatment with x ray and urethan and died less than one year after symptoms first appeared

Case 2

A 71 year old white ship inspector was in excellent health until six months prior to his admission when he had a febrile illness associated with malaise The fever subsided within a week or ten days but weakness continued three months later the patient again developed fever accompanied by a non productive cough and pain in the right chest that was aggravated by coughing Roentgenogram of the chest at that time revealed infiltration of the right lung The pain and fever subsided and the lesion in the right lung cleared but anorexia mid epigastric discomfort and loss of weight continued until admission

When admitted to the hospital the patient appeared slightly pale and chronically ill but the physical examination revealed no other significant abnormalities

Laboratory Examination Urinalysis specific gravity 1 020 albumin trace otherwise negative (no Bence Jones protein was demonstrated) hematocrit 29 per cent hemoglobin 9 0 gm per 100 ml rbc 3 2 mil per

cu mm white count 3800 per cu mm differential count (per cent) segmented 46 bands 2 lymphocytes 32 monocytes 18 eosinophils 2 The serum albumin was 3 0 gm per 100 ml and the serum globulin 6 8 gm per 100 ml Blood urea was 34 mg and the uric acid 7 3 mg per 100 ml Bone marrow obtained by aspiration consisted almost entirely of cells of the plasmacytic series 98 per cent of the cells were of this type In some the nuclear chromatin was finely divided and nucleoli were present Roentgenograms of the skeleton revealed no significant lesions either lytic or osteoporotic

Course A diagnosis of multiple myeloma was made Urethan was administered in an initial dose of 0 3 gm three times a day the dose was increased gradually to a total of 4 0 gm per day The patient also received blood transfusions The symptoms subsided but improvement was short lived About a month after leaving the hospital he developed severe girdle pain which was rapidly followed by paraplegia Laminectomy was performed but the patient did not improve He died eight months after the onset of symptoms

Comment This patient was elderly at the time of onset of symptoms and survived for less than one year His illness was characterized by pain normocytic anemia hyperproteinemia and great numbers of plasma cells in the bone marrow An onset with symptoms that suggest recurrent pulmonary infections occurs in a number of patients with myeloma but as was true with this patient diagnosis of multiple myeloma was not suspected The course of the disease in this patient was unusual in that roentgenograms of the spine revealed no significant abnormalities only a few months before compression fracture of a vertebra and paraplegia occurred As is true in about half the patients with this disease Bence Jones protein was not demonstrated in the urine by the usual tests it can be demonstrated in nearly all cases by special tests

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that is withdrawn are usually best because the specimen is largely undiluted with blood. Some hematologists prefer to withdraw a larger specimen from the marrow and eject the material from the syringe into a watch glass. Particles of marrow are then picked up on an applicator stick or some other instrument and touched to various areas of the clean slide. Such imprints give a better picture of the marrow architecture and of the fixed cells. However, preparations made in this manner usually contain a large number of

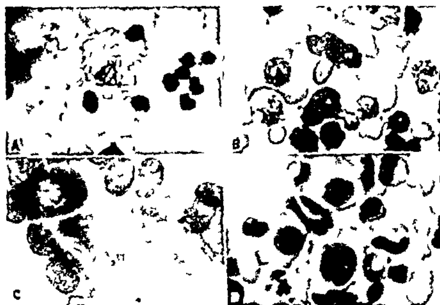


Figure 68. Bone marrow aspiration. Wright's stain in various diseases. A, Cirrhosis. B, Reticulum cell sarcoma. C, Metastatic carcinoma. D, Hodgkin's sarcoma.

damaged and unrecognizable cells. Bone marrow obtained by aspiration can be stained supravitally, with Wright's stain, with May-Grunwald-Giemsa stain, or by the peroxidase method. Marrow particles obtained by aspiration can also be fixed in paraffin and stained with hematoxylin and eosin.

A differential count is often performed on the nucleated cells in the marrow preparation. For a reproducible result it is usually necessary to count from 300 to 500 cells. The range of the different cells varies in normal marrows and the values given by different authors differ somewhat, as shown in Table 20. An accurate differential count is helpful in diagnosis on some occasions. It is also an excellent discipline for those in training because it forces a decision as to the identity of each cell. However, it is time consuming and after a moderate amount of experience it is usually possible to gain the same information from a careful systematic study of the smear without actually doing a differential count.

In examining the bone marrow it is desirable to follow a definite plan

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i classed as normal or abnormal and the abnormality is specified The megakaryocytes and platelet formation are classed as normal reduced or increased Similarly the number and maturity of the lymphocytes plasma cells and reticulum cells are recorded These observations form the basis for the conclusions that can be drawn from the marrow examination

The Value of Bone Marrow Examination

Examination of the bone marrow may establish a diagnosis of certain diseases with assurance it may render some particular diagnosis very unlikely or it may give helpful information that is of indirect or limited value The ease with which bone marrow can be obtained for examination has led to the use of this procedure in circumstances when other simpler procedures such as a careful study of a smear of peripheral blood would suffice for a diagnosis The decision as to the desirability of a bone marrow examination is based on the circumstances in the individual case

Although the marrow examination may not be necessary in order to establish the diagnosis the following diagnoses can usually be made by this method

1 Multiple myeloma
2 Leukemia, all types
3 Untreated or partially treated vitamin B_{12} or folic acid deficiency
4 Storage disease such as Gaucher's disease
5 Metastatic malignancy (if positive cells are found)
6 Fungus disease (if positive cells are found)

In certain other diseases when taken in conjunction with the peripheral blood the bone marrow examination can often provide helpful information that is not diagnostic when taken alone Among these are

1 Aplastic anemia
2 Idiopathic thrombocytopenic purpura
3 Lymphosarcoma
4 Hypersplenism
5 Agranulocytosis

It is sometimes difficult to exclude a diagnosis from further consideration because of the result of the bone marrow examination Myeloma cells" or a definite increase in plasma cells may not be found in multiple myeloma Some patients with aplastic" anemia have cellular marrows Patients with pernicious anemia sometimes forget to relate that they have taken vitamin tablets that contain folic acid or have received an injection of vitamin B_{12} from another physician Aspiration occasionally yields only a hypocellular or aplastic marrow in patients with leukemia When the diagnosis remains in doubt and only very hypocellular preparations are obtained from different sites a surgical biopsy of the marrow is usually obtained If abnormal bleeding is expected with this procedure the biopsy is best performed in the iliac crest because bleeding is controlled at this site more easily than in the sternum or rib

Table 20 Normal Adult Differential Marrow Cell Counts by Various Authors

CELL TYPE	WINTROBE (%) (RANGE)	CUSTER (%) (RANGE)	ISRAELS[3] (%) (RANGE)	LEITNER[4] (%)	YOUNG AND OSGOOD[6] (%) (RANGE)
Myeloblasts	0 3– 5 0	0 0– 3 5	0 3– 2 0	1 2	0 0– 1 2
Progranulocytes	1 0– 8 0	0 5– 5 0	1 0– 8 0	2 2	0 0– 7 8
Myelocytes					
Neutrophils	5 0–18 0	7 0–34 6	5 0–20 0	12 6	0 0– 2 6
Eosinophils	0 5– 3 0	0 3– 3 0		1 4	0 0– 0 4
Basophils	0 0– 0 5	0 0– 0 5		0 02	
Metamyelocytes	13 0–32 0	15 1–37 0	13 0–32 0	11 0	1 8–11 8
Band Cells				24 0	15 8–33 0
Segmented					
Neutrophils	7 0–30 0	3 0–19 8	7 0–30 0	28 4	7 4–25 2
Eosinophils	0 5– 4 0	0 1– 3 0	0 5– 4 0	1 8	0 0– 1 0
Basophils	0 0– 0 7	0 0– 1 0	0 0– 1 0	0 02	0 0– 0.2
Lymphocytes	3 0–17 0	0 0– 6 8	3 0–20 0	7 6	4 8–16 0
Plasmacytes	0 0– 2 0	0 0– 1 2	0 0– 2 0	1 0	0 0– 1 0
Monocytes	0 5– 5 0	0 1– 3 2	0 5– 5 0	1 4	0 0– 4.2
Reticulum Cells	0 1– 2 0	0 3– 2 6		2 0	
Megakaryocytes	0 03–3 0	0 05–1 5	Occas	0 8	0 0– 0 2
Pronormoblasts	1 0– 8 0	0 0– 0 0	0 5– 4 0	0 8	
Normoblasts (basophilic polychromatic and orthochromatic)	7 0–32 0	17 5–42 0	19 0–35 0	27 6	5 4–24 2
Myeloid Erythroid Ratio	about 3 1– 4 1	2 1– 6 1	4 1–20 1	3 1	2 1– 8 1

The slide is first examined with the low power objective The cellularity of the specimen is noted the number of megakaryocytes is estimated and a search for sheets of malignant cells and other unusual cells is made particularly near the edges of the preparation The more suitably stained portions of the slide are examined under oil immersion The erythrocyte precursors are studied and the type of erythropoiesis is noted as megaloblastic or normoblastic Attention is also paid to the number and maturity of these cells Next the granulocytic series is studied with particular reference to number and any increase in the younger forms particularly myeloblasts and progranulocytes The presence of any macropolycytes or giant metamyelocytes is also noted Megakaryocytes are studied for number maturity and evidence of platelet formation The plasma cells reticulum cells histiocytes and other cells are studied similarly for number and immaturity and for cytoplasmic inclusions The number of lymphocytes is estimated and the presence of any tumor cells or other abnormal or unidentified cells is noted.

After the marrow preparation has been studied in this fashion the different observations can be noted in the report The cellularity is classed as normal *increased or decreased Erythropoiesis is classified according* to the type of maturation as normoblastic or megaloblastic and according to activity as normal hypoplastic or hyperplastic The granulocytic maturation

0.5 gm mercuric chloride
5.0 gm sodium sulfate
1.0 gm sodium chloride
200 ml distilled water

2. Hemacytometer and red blood cell diluting pipette

Method

1. Either freshly collected heparinized venous blood (7.5 mg heparin/5 ml blood) or free flowing blood from a finger puncture may be used. If heparinized blood is used, it should be mixed well by inverting the container 20 to 30 times.

2. Draw blood up to the 0.5 mark of the erythrocyte counting pipette. The pipette should be clean and dry. Wipe all blood from the outside of the pipette and make needed adjustments in the height of the column of blood within the pipette by touching the end to blotting paper or cotton sponge.

3. Keeping the pipette as nearly horizontal as possible, draw Hayem's solution into the pipette until the column reaches the 101 mark (1 to 200 dilution).

4. Shake pipette and contents by hand or with mechanical shaker for one minute.

5. Expel ⅓ of contents and immediately thereafter load both sides of the counting chamber by placing a drop of cell suspension on the chamber at the edge of the cover glass. The suspension fills the ruled area by capillary attraction.

6. Should the chamber flood or bubbles occur, the results will be inaccurate. The chamber must then be cleaned and reloaded.

7. Allow three minutes for settling, then count the cells in the 16 smallest squares (0.0025 mm^2 each) of three corners of two sides of the chamber. Discard the first count and add the other five. Multiply by 10,000 to obtain the number of red blood cells per cu mm.

Calculation (Reference 2)

The dilution of the blood with Hayem's solution is 1:200. The distance between the cover slip and the chamber is 0.1 mm; the smallest squares have an area of 0.0025 sq mm and 80 of them are counted. The total area in which cells are counted is 0.2 mm^2 and the volume (0.2 mm^2 × 0.1 mm) is 0.02 cu mm. To determine the number of cells in 1 cu mm, the number of cells found in 0.02 cu mm must be multiplied by 50 (1/0.02). The result is then multiplied by 200 to correct for dilution.

The factor then is 50 × 200 = 10,000. If the number of red blood cells counted in a total area of 0.02 cu mm is multiplied by 10,000, the result will be the number of red blood cells per cubic millimeter of whole blood.

LABORATORY PROCEDURES IN HEMATOLOGY

HEMATOCRIT

Method (Reference 1)

1 Collect 5 ml of venous blood with a dry syringe and needle Place in a small bottle containing 7 5 mg heparin and mix well by inverting bottle at least 20 times

2 Fill a Wintrobe hematocrit tube to the 10 cm mark with fresh heparinized blood using a Wintrobe pipette

3 Centrifuge at 3000 r p m for 30 minutes

4 The volume of packed red cells multiplied by 10 gives the volume per 100 ml blood

Calculations

Normal Females 42 0 ± 5 0
Males 47 0 ± 7 0

Note

It should be noted that marked alteration in the tonicity of the plasma will alter the volume of the red blood cell The hematocrit is a more accurate procedure than the red blood cell count and in many laboratories has replaced the latter as a routine procedure This method is accurate within 0 5 per cent (reference 2)

References

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2 Wintrobe M M Blood of normal young women residing in a sub tropical climate Arch Int Med *45* 287 1930

ERYTHROCYTE COUNT

Equipment

1 Hayem s Solution

0 5 gm mercuric chloride
5 0 gm sodium sulfate
1 0 gm sodium chloride
200 ml distilled water

2. Hemacytometer and red blood cell diluting pipette

Method

1 Either freshly collected heparinized venous blood (7 5 mg heparin/5 ml blood) or free flowing blood from a finger puncture may be used If heparinized blood is used it should be mixed well by inverting the container 20 to 30 times

2. Draw blood up to the 0 5 mark of the erythrocyte counting pipette The pipette should be clean and dry Wipe all blood from the outside of the pipette and make needed adjustments in the height of the column of blood within the pipette by touching the end to blotting paper or cotton sponge

3 Keeping the pipette as nearly horizontal as possible draw Hayem's solution into the pipette until the column reaches the 101 mark (1 to 200 dilution)

4 Shake pipette and contents by hand or with mechanical shaker for one minute

5 Expel ⅓ of contents and immediately thereafter load both sides of the counting chamber by placing a drop of cell suspension on the chamber at the edge of the cover glass The suspension fills the ruled area by capillary attraction

6 Should the chamber flood or bubbles occur the results will be inaccurate The chamber must then be cleaned and reloaded

7 Allow three minutes for settling then count the cells in the 16 smallest squares (0 0025 mm each) of three corners of two sides of the chamber Discard the first count and add the other five Multiply by 10 000 to obtain the number of red blood cells per cu mm

Calculation (Reference 2)

The dilution of the blood with Hayem's solution is 1 200 The distance between the cover slip and the chamber is 0 1 mm the smallest squares have an area of 0 0025 sq mm and 80 of them are counted The total area in which cells are counted is 0 2 mm and the volume (0 2 mm $\times$ 0 1 mm) is 0 02 cu mm To determine the number of cells in 1 cu mm the number of cells found in 0 02 cu mm must be multiplied by 50 (1/0 02) The result is then multiplied by 200 to correct for dilution

The factor then is $50 \times 200 = 10\,000$ If the number of red blood cells counted in a total area of 0 02 cu mm is multiplied by 10 000 the result will be the number of red blood cells per cubic millimeter of whole blood

LABORATORY PROCEDURES IN HEMATOLOGY

HEMATOCRIT

Method (Reference 1)

1 Collect 5 ml of venous blood with a dry syringe and needle Place in a small bottle containing 7 5 mg heparin and mix well by inverting bottle at least 20 times

2 Fill a Wintrobe hematocrit tube to the 10 cm mark with fresh heparinized blood using a Wintrobe pipette

3 Centrifuge at 3000 r p m for 30 minutes

4 The volume of packed red cells multiplied by 10 gives the volume per 100 ml blood

Calculations

Normal	*Females*	$42\,0 \pm 5\,0$
	Males	$47\,0 \pm 7\,0$

Note

It should be noted that marked alteration in the tonicity of the plasma will alter the volume of the red blood cell The hematocrit is a more accurate procedure than the red blood cell count and in many laboratories has replaced the latter as a routine procedure This method is accurate within 0 5 per cent (reference 2)

References

1 Wintrobe M M Macroscopic examination of the blood Am J M Sc *185* 58 1933
2 Wintrobe M M Blood of normal young women residing in a sub tropical climate Arch Int Med *45* 287 1930

ERYTHROCYTE COUNT

Equipment

1 Hayem s Solution

0.5 gm mercuric chloride
5.0 gm sodium sulfate
1.0 gm sodium chloride
200 ml distilled water

2. Hemacytometer and red blood cell diluting pipette

Method

1. Either freshly collected heparinized venous blood (7.5 mg heparin/5 ml blood) or free flowing blood from a finger puncture may be used. If heparinized blood is used, it should be mixed well by inverting the container 20 to 30 times.

2. Draw blood up to the 0.5 mark of the erythrocyte counting pipette. The pipette should be clean and dry. Wipe all blood from the outside of the pipette and make needed adjustments in the height of the column of blood within the pipette by touching the end to blotting paper or cotton sponge.

3. Keeping the pipette as nearly horizontal as possible draw Hayem's solution into the pipette until the column reaches the 101 mark (1 to 200 dilution).

4. Shake pipette and contents by hand or with mechanical shaker for one minute.

5. Expel ⅓ of contents and immediately thereafter load both sides of the counting chamber by placing a drop of cell suspension on the chamber at the edge of the cover glass. The suspension fills the ruled area by capillary attraction.

6. Should the chamber flood or bubbles occur the results will be inaccurate. The chamber must then be cleaned and reloaded.

7. Allow three minutes for settling, then count the cells in the 16 smallest squares (0.0025 mm² each) of three corners of two sides of the chamber. Discard the first count and add the other five. Multiply by 10,000 to obtain the number of red blood cells per cu mm.

Calculation (Reference 2)

The dilution of the blood with Hayem's solution is 1:200. The distance between the cover slip and the chamber is 0.1 mm; the smallest squares have an area of 0.0025 sq mm and 80 of them are counted. The total area in which cells are counted is 0.2 mm², and the volume (0.2 mm² × 0.1 mm) is 0.02 cu mm. To determine the number of cells in 1 cu mm, the number of cells found in 0.02 cu mm must be multiplied by 50 (1/0.02). The result is then multiplied by 200 to correct for dilution.

The factor then is 50 × 200 = 10,000. If the number of red blood cells counted in a total area of 0.02 cu mm is multiplied by 10,000 the result will be the number of red blood cells per cubic millimeter of whole blood.

Note

With so large a factor there is little wonder that the accuracy of the red blood cell count is so poor The standard error for a single erythrocyte count has been determined to be 7 8 per cent (reference 1) Such an error is even more significant when compounded by the calculation of indices

References

1 Berkson J Magath T B and Hurn M The error of estimate of the blood cell count as made with the hemocytometer Am J Physiol *128* 309 1940
2 Bray W E Clinical laboratory methods C V Mosby Co St Louis 1957

TOTAL HEMOGLOBIN (CYANMETHEMOGLOBIN METHOD)

Reagents and Equipment

1 Drabkin s Solution (Diluent)

$NaHCO_3$	1 gm
KCN	52 mg
$K_3Fe(CN)_6$	198 mg
Distilled water	To make 1 liter

While there is but a small amount of cyanide in this solution and it would take some 4 liters to be lethal to man it is a poison and should be handled with care Inhalation of fumes should be avoided Any of the salts spilled during preparation should be wiped up with a damp cloth and disposed of in a suitable container Pipettes should be filled by using suction bulbs to avoid the possibility of getting the solution in the mouth The salts should be kept in a locked cupboard The solution should be stored in a brown bottle in the refrigerator (reference 2)

2 Standardized cuvettes
3 Standardized blood diluting pipettes (Sahli 0 02 ml)
4 Cyanmethemoglobin standards *
5 Spectrophotometer or colorimeter

Method

1 Exactly 5 ml of Drabkin s solution is measured into each of two matched cuvettes

2 To one tube 0 02 ml of whole blood is added the pipette rinsed and the blood and solution mixed by swirling

3 Allow to stand 10 minutes

* Standards may be obtained from the College of American Pathologists 203 North Wabash Avenue Chicago Illinois

4. Using the blank tube set the spectrophotometer at 540 wave length and adjust to 100 per cent light transmittance

5 Substitute the tube containing hemoglobin solution and read the per cent of light transmitted

6 Multiply the value by the conversion factor of the pipette

7 The result is translated into grams per 100 ml blood by reference to standard graph

Note

In view of the many known physical and chemical reactions of hemo globin it is strange that a completely satisfactory standard has not been developed Poor stability of the solutions employed and difficulty of match ing colors whether by mechanical or visual means have plagued all methods

The basic reaction in the production of the cyanmethemoglobin stand ard is the conversion of hemoglobin to methemoglobin by ferricyanide and the subsequent combination of methemoglobin with potassium cyanide to form the pigment cyanmethemoglobin Solutions of this pigment are rela tively stable After 3 years storage at refrigerator temperatures the standards faded only 1 per cent (reference 3) Stored at room temperature the stand ard faded more rapidly and 3 per cent was lost.

After preliminary trials by the Army Medical Service Graduate School the National Academy of Sciences and the National Research Council pro posed the adoption of a National Standard for hemoglobinometry (refer ence 1) The method recommended was the cyanmethemoglobin one Should the program prove successful truly comparable hemoglobinometry will be possible for the first time

Macfarlane et al (references 4 and 5) have reviewed extensively the problems attending hemoglobinometry and the reader interested in such a review is referred to their excellent articles

References

1 Cannan R K Proposal for the distribution of a certified standard for use in hemoglobinometry Am J Clin Path 25 376 1955

2 Crosby W H Munn J I and Furth F W Standardizing a method for clinical hemoglobinometry U S Armed Forces Med J 5 693 1954

3 Crosby W H and Houchin D N Preparing standard solutions of cyanmethemoglobin Blood 12 1132 1957

4 King E J et al Determination of haemoglobin V Precision of colori metric methods Lancet 2 563 1948

5 Macfarlane R F King E J Wootton I D P and Gilchrist, M Determination of haemoglobin III Reliability of clinical and other methods Lancet, 1 282 1948

LEUKOCYTE COUNT

(Reference 2)

Reagents and Equipment

1 0 1 N hydrochloric acid

2 Hemacytometer and white blood cell diluting pipette

Method

1 Either freshly collected heparinized venous blood or free flowing blood from a finger puncture may be used If heparinized blood is used mix well by inverting container 20 to 30 times

2 Draw blood up to the first mark in the leukocyte counting pipette Wipe blood from outside of pipette and make necessary adjustments in the column of blood by touching the end of the pipette to blotting paper or cotton sponge

3 Keeping pipette as nearly horizontal as possible draw sufficient 0 1 N hydrochloric acid into the pipette to raise the column of liquid to the 11 mark (1 20 dilution)

4 Shake pipette by hand or mechanical shaker for one minute

5 Expel ⅓ of the contents and immediately thereafter load the counting chambers by placing a drop of cell suspension on the chamber at the edge of the cover slip

6 Should the chamber flood or bubbles occur the results will be in accurate The chamber must be cleaned and reloaded

7 Allow three minutes for settling and count the cells in three corner square millimeter areas on each of the two sides of the preparation Obtain the average and multiply by 200 to determine the number of leukocytes per cu mm of whole blood

Calculations

The total area over which the leukocytes are counted is 5 mm The distance between the cover slip and the chamber is 0 1 mm and the dilu tion of blood is 1 20

The number of cells in five of the 1 mm^2 squares is counted and the average number is multiplied by 0 1 to obtain the number of leukocytes in 0 1 mm^3 and by 10 to determine the number of cells in 1 0 cu mm The result is then multiplied by 20 to correct for dilution

The factor is then $10 \times 20 = 200$ Multiplication of the number of leukocytes counted in the average 1 mm^2 area gives the number of leuko cytes in one cubic millimeter of whole blood

Note

The error in leukocyte counting is approximately 10 per cent (reference 1) If the count is very high as in leukemia the dilution should be made

in an erythrocyte counting pipette and appropriate corrections made in the calculations

References

1 Berkson J Magath T B and Hurn M The error of estimate of the blood cell count as made with the hemocytometer Am J Physiol 128 309 1940
2 Bray W E Clinical Laboratory Methods C V Mosby Co St Louis 1957

STAINING OF PREPARED BLOOD FILMS

Reagents

1 Wrights stain

0 2 gm dry Wrights stain
97 0 ml methanol C P A C S Acetone free
3 0 ml glycerol C P

Mix alcohol and glycerol add stain
Allow to stand at least 24 hours with occasional shaking A longer period of aging is desirable for consistent staining results
Filter before using

Method

1 Fresh smears are preferable
2 Place the air dried blood film on a level support
3 Completely cover the blood smear with stain and allow to remain for one minute This serves to fix the smear
4 The stain is then diluted with an equal amount of distilled water
5 After five or six minutes the stain is washed off with water and the smear is blotted gently or is allowed to dry

DIFFERENTIAL COUNT

Reagent

1 Wrights stain (see previous procedure)

Method

1 The smear is made by placing a medium sized drop of blood near one end of a clean dry glass slide and immediately smearing it along the length of the slide by means of a second slide tilted at a 45 degree angle
2 The smear is allowed to dry and is stained with Wrights stain
3 The desired number (usually 100 to 200) of leukocytes are counted on the thinnest portion of the smear

Calculation

Schilling's hemogram for normal blood

0	Myelocytes
0–1%	Juveniles
3–5%	Bands
58–66%	Segmented cells
21–35%	Lymphocytes
4–8%	Monocytes
2–4%	Eosinophils
0–1%	Basophils

The number of platelets is estimated as normal increased" or decreased"

The red cells are studied for the presence or absence of anisocytosis poikilocytosis and polychromatophilia hypochromia stippling and other abnormalities They should be studied only in those areas of the slide where they just touch one another

Note

Only practice will enable the operator to make smears which are satisfactory Clean dry slides are imperative It is helpful to remove the corners of the slide used to smear the blood by rubbing them against a piece of screen mesh This keeps the smear from extending to the edge of the slide in preparation Swift motion is not necessary but a smooth even pace with gradual release of pressure will result in a smooth smear and a feather edge

WHITE BLOOD CELL CONCENTRATION

Apparatus

1 Clean centrifuge tube or hematocrit tube

Method

1 Fill tube with fresh anticoagulated blood

2 Centrifuge at low speed for 5 minutes then at high speed for 10 minutes or until the white cell layer is clearly defined

3 Pipette off the plasma and smear the white cell layer

4 Stain with Wright's stain

Note

Such a preparation while useful in searching for immature cells is not suitable for a differential count because of the uneven distribution of the cells

If the patient's white count is extremely low a larger amount of blood may be centrifuged, the white cell layer placed in a hematocrit tube and recentrifuged

SEDIMENTATION RATE

Method

1 Five ml of venous blood is collected with a dry syringe and needle and mixed in a small bottle containing 4 mg solid potassium oxalate and 6 mg solid ammonium oxalate as an anticoagulant

2 This blood should be used for the determination of sedimentation rate within two hours of its time of collection

3 Fill a hematocrit tube to the 10 cm mark The tube should be kept in an exact vertical position during the sedimentation of the blood corpuscles and the room temperature should be not less than 24° C nor more than 27° C

4 Read the distance the corpuscles have fallen in one hour

Calculation

Normal Females 0 to 15 mm /one hour
Males 0 to 6 5 mm /one hour

Note

The rate at which blood sediments depend for the most part on the concentration of fibrinogen in the plasma Variation in the concentration of other globulins will result in an altered sedimentation rate as well Alterations of these proteins during the course of infection is probably responsible for the elevated sedimentation rate

Reference

1 Wintrobe M M and Landsberg J W A standardized technique for the blood sedimentation test Am J M Sc 189 102 1935

STAIN FOR RETICULOCYTES

Reagents

1 Brilliant Cresyl Blue
1 3 gm sodium oxalate
1 0 gm brilliant cresyl blue
100 ml distilled water
Let stand a few days and filter
Store in dark bottle

Method

1 Use either fresh anticoagulated (heparin or oxalate) venous blood or blood from finger puncture

2 On a clean dry slide place a small drop of blood and an equal amount of brilliant cresyl blue staining solution Mix with the corner of another slide and allow to stand two or three minutes

3 Mix again and make a fairly thin smear

4 When smear is dry counterstain with Wright s stain

5 Count the number of reticulated red cells per thousand red cells and express as the number of reticulated cells per 100 red cells (per cent)

Note

The normal number of reticulocytes is from 0 1 to 1 0 per cent They are increased at times of rapid regeneration and release of red cells from the marrow

References

1 Osgood E E and Wilhelm M M Reticulocytes J Lab & Clin Med *19* 1129 1934

DEMONSTRATION OF THE LUPUS ERYTHEMATOSUS CELL PHENOMENON

Reagents and Equipment

1 Clean dry sterile test tube

2 3 x 3 cm square piece of wire mesh

3 Wright s stain

Method

1 10 ml of blood is placed in clean dry sterile test tube and allowed to clot at room temperature of 28 C or in a water bath at 37° C for 2 hours

2 The serum is discarded and the clot is forced through the mesh screen to defibrinate it

3 The fluid portion of the clot is collected in a clean sterile Petri dish and then transferred to a small test tube or hematocrit tube for centrifugation

4 Tubes are centrifuged at 1500 to 1800 r p m for five minutes

5 Serum is discarded and the buffy layer is collected and a drop is placed on a clean dry slide and smeared in usual fashion

6 Stain with Wright s stain

Note

The L. E. cell is defined as a phagocytic cell that contains an inclusion body consisting of nuclear material that has undergone lysis. The phagocytic cell is usually the polymorphonuclear leukocyte but eosinophils may also play this role. *The inclusion body is a smooth homogeneous nuclear mass* which has been released from a neutrophil or lymphocyte affected by the *L.E. factor (reference 2). In normal blood no typical L.E. cells are found.* The L.E. cell is formed when the polymorphonuclear leukocyte engulfs an amorphous nuclear remnant. *The "tart cell" differs from the L.E. in that* some structure may be seen in the nuclear remnant which has been engulfed.

A "rosette" consists of a group of polymorphonuclear leukocytes which have surrounded an amorphous protein mass. The finding of "tart" cells, "rosettes" or a free amorphous mass of protein is not "positive" of the L.E. phenomenon.

The phenomenon is not seen in smears made directly after the aspiration of blood or bone marrow because a period of incubation in vitro is required (reference 2). The lupus cell phenomenon probably depends on a reaction between the L.E. factor found in the serum of patients with lupus erythematosus and the cell nucleus. This reaction results in the morphologic changes in the nuclear material. The second step is that of phagocytosis. Evidence has been presented that a heat labile factor present in normal serum is required for the phagocytosis. The L.E. factor does not have to be present for the ingestion of the altered nuclear material (reference 1).



Figure 69. L. E. cells from peripheral blood of a patient with disseminated lupus erythematosus.

References

1 Aisenberg A C Studies on the mechanism of the lupus erythematosus (L E) phenomenon J Clin Invest 38 325 1959
2 Hargraves M M The L E Cell Phenomenon In Dock W and Snapper I Eds Advances in Internal Medicine Vol 6 Year Book Publishers Chicago 1954 p 133

PEROXIDASE STAIN FOR LEUKOCYTES

Reagents

1 0 3 gm benzidine base
99 0 ml ethyl alcohol
Grind adding a little of the alcohol at a time When dissolved add
2 Sodium nitroprusside 1 0 ml (saturated 36%)
This solution keeps several months
3 Hydrogen peroxide 3% (fresh) 5 drops in 25 ml water Make fresh each test
4 Wright s stain

Method

1 Use fresh blood smears either from finger puncture or from venous blood collected in heparin

2 Place about 10 drops of benzidine solution on the smear and let stand 1 to 1½ minutes

3 Without pouring off the benzidine solution add 5 drops of freshly diluted hydrogen peroxide (5 drops in 25 ml of water) and let stand three or four minutes

4 Wash with water and allow to dry

5 Stain with Wright s stain

Note

This stain is used to distinguish the myelocytic cells from the other leukocytes The myelocytic cells show large blue green granules with the eosinophils being more deeply blue The cells of the lymphocyte series and myeloblasts do not take the stain Monocytes may show a few small irregular and discrete granules Basophiles are peroxidase negative

References

1 Osgood E E and Ashworth C M Atlas of Hematology J W Stacey Inc San Francisco 1937 p 206
2 Washburn A H A combined peroxidase and Wright s stain for routine blood smears J Lab & Clin Med 14 246 1928

LEUKOCYTE ALKALINE PHOSPHATASE STAIN

I MODIFICATION OF GOMORI PROCEDURE

(References 1 and 4)

Reagents

A Stock
1 2% Calcium chloride solution
2 3% Sodium β glycerophosphate solution
3 10% Magnesium sulfate solution
4 10% Sodium barbital solution
5 1% Cobalt nitrate solution
These solutions are stable but should be stored in refrigerator to discourage mold growth
6 Dilute ammonium sulfide solution

B Incubating Solution
5–10 ml 3% sodium β glycerophosphate solution
20–25 ml 2% calcium chloride solution
10 gtt 10% magnesium sulfate solution
10 ml 10% sodium barbital solution
Distilled water to 50 ml total volume
The incubation solution must be made fresh daily

Method

1 Fix smears of peripheral blood or bone marrow in 95% ethyl alcohol within 30 minutes of their preparation Dry

2 Place slides in incubation solution at 37 for 1 to 1½ hours Remove and wash in 2% calcium chloride solution Dry

3 Immerse slides for 5 minutes in 1% cobalt nitrate solution Wash in tap water and dry

4 Immerse slides for 5 minutes in dilute ammonium sulfide solution Wash in tap water and dry

5 Counter stain with Wright s stain

Calculations

1 Count 100 neutrophils assigning to each a score

0 – no stain
± – faint gray tinge in cytoplasm
1+ – distinct but infrequent granules
2+ – granules more dense
3+ – intensity of staining and number of granules greater
4+ – densely stained cytoplasm

2 Control slide should be run with each unknown

II AZO DYE METHOD
(References 2 and 3)

Reagents

STOCK PROPANEDIOL BUFFER *0 2 M*
10 5 gm 2 amino 2 methyl propane (1 3) diol
500 ml distilled water

WORKING BUFFER *0 05 M pH 9 75*
25 ml stock buffer
5 ml 0 1 N hydrochloric acid
70 ml distilled water

SUBSTRATE MIXTURE
35 mg sodium α naphthyl phosphate
35 mg Brentamine Fast Garnet
35 ml working 0 05 M propanediol buffer
This mixture is made up fresh each time the procedure is done and should be used within 3 minutes of preparation

FIXATIVE
10 ml 40% formaldehyde
90 ml absolute methyl alcohol
Keep in refrigerator at 0 to 5 C

COUNTERSTAIN
2 gm methyl green
100 ml distilled water

After the methyl green has gone into solution approximately one half of its volume of chloroform is added The chloroform extracts methyl violet impurities from the methyl green After 48 hours the solution is poured into a separatory funnel The chloroform settles to the bottom and can be run off and discarded The supernatant fluid is then ready for use

Method

Blood and bone marrow smears for this purpose should be stained on the day of collection and preferably fixed within an hour of collection

1 Fix smears for 30 seconds at 0 to 5° C in formaldehyde–methyl alcohol solution

2 Wash in tap water

3 Make up substrate solution mix and filter directly onto the slides

4 Leave at room temperature for 5 to 10 minutes

5 Wash in tap water for 10 seconds

6 Counterstain with methyl green for 10 to 15 minutes

7 Wash in tap water

8 Dry and mount in glycerin jelly or other water soluble mount Mounting is necessary as smears fade after 2 or 3 days

9 Examine under oil Red cells and nuclei are stained green Alkaline phosphatase stains are brown black granules within the cytoplasm of the neutrophils

Calculation

Proceed to examine 100 neutrophils assigning to each the following score

0—negative or colorless
1—occasional small granules
2—moderate granule formation
3—intense granule formation
4—dense precipitate of granules filling whole cytoplasm of cell

Add up the scores for the 100 individual cells normal range 14–100

Note

The role of alkaline phosphatase activity in the leukocyte remains a mystery The significance of the difference in the alkaline phosphatase activity of polymorphonuclear leukocytes in chronic myelogenous leukemia and leukemoid reactions is not understood However these differences are striking and have been exploited by the tests described Three major categories may be defined

1 Low Range	0–24 total score
a Chronic myelogenous leukemia	
2 Normal Range	14–100 total score
3 High Range	100–400 total score

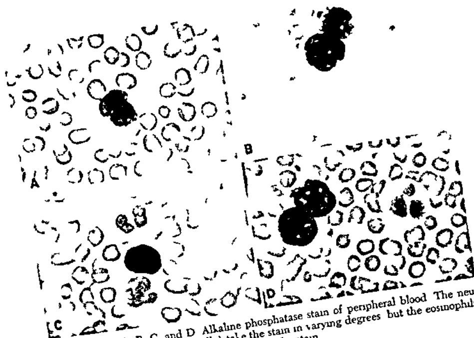


Figure 70 A B C and D Alkaline phosphatase stain of peripheral blood The neutrophils (band and segmented cells) take the stain in varying degrees but the eosinophils monocytes and lymphocytes are negative for the stain

a Leukemoid reaction
b Infectious leukocytosis
c Polycythemia
d Hodgkin s disease
e Myelofibrosis

While there is an overlap between the normal range and that of chronic myelogenous leukemia, there is none between chronic myelogenous leukemia and leukemoid reactions It is in the differentiation of these conditions that the test is most helpful

References

1 Brodell H and Swisher S N Studies of leukocyte alkaline phosphatase determined by a clinically applicable histochemical method Clin Res Proc *2* 58 1954
2 Hayhoe F G J and Quaglino D Cytochemical demonstration and measurement of leukocyte alkaline phosphatase activity in normal and pathological states by a modified azo dye coupling technique Brit J Haemat *4* 375 1958
3 Kaplow L S A histochemical procedure for localizing and evaluating leukocyte alkaline phosphatase activity in smears of blood and marrow Blood *10* 1023 1955
4 Wiltshaw E and Moloney W C Histochemical and biochemical studies on leukocyte alkaline phosphatase activity Blood *10* 1120 1955

SICKLE CELL TEST

Reagents

1 Two per cent sodium bisulfite solution 500 mg sodium bisulfite is dissolved in distilled water the total volume being brought to 25 ml As the solution must be made fresh it is convenient to store 500 mg lots of sodium bisulfite in large capsules until needed

Method

1 The preparations may be made directly from a finger puncture or freshly drawn anticoagulated blood

2 Equal amounts of blood and sodium bisulfite are mixed on a glass slide and covered with a cover slip Slight pressure on the cover slip will rid the preparation of air bubbles and the desired thinness may be obtained

3 Inspect the preparation after 10 to 15 minutes for sickled cells The number of sickled red cells per 1000 red blood cells are determined and expressed in per cent

Note

Sickling is the result of the formation of rigid tactoids of hemoglobin molecules These structures distort the stroma and result in the formation

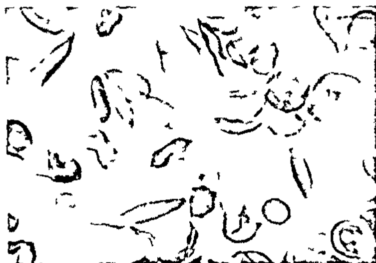


Figure 71 Moist preparation sickle cell anemia

of the classic sickle cell At least 20 per cent of the hemoglobin must be of the "S" type for sickling to occur Because of the high percentage of fetal hemoglobin present at birth blood cells from infants seldom sickle By the age of 4 to 6 months the level of fetal hemoglobin falls and is replaced by one of the adult forms of hemoglobin If that form of hemoglobin is of the "S" type as its percentage increases sickling of the erythrocytes will be seen

All of the cells from a patient with A S hemoglobin (heterozygote) will sickle if the blood is left long enough without oxygen While there are some morphologic differences between sickle cells of the heterozygote and homozygote they are not consistent enough to be diagnostic

Reference

1 Daland G A and Castle W B A simple and rapid method for demonstrating sickling of the red blood cells The use of reducing agents J Lab & Clin Med 33 1082 1948

OSMOTIC FRAGILITY

Reagents and Equipment

1 A chemically pure 0 75 per cent solution of sodium chloride is required. The saline solution must be kept in a glass stoppered tightly closed bottle

2 Eighteen small test tubes (Wassermann tubes)

Method

1 Eighteen small test tubes are set up in a rack and numbered from left to right 48 46 44 through 14

2 Into the first tube is measured 4 8 ml of the sodium chloride solution into the second 4 6 ml and so on in amounts corresponding to the numbers on the tubes

3 To each tube there is added the amount of distilled water required to bring the total in each tube to 5 ml

4 The concentration of sodium chloride in each tube will be from left to right 0 72 per cent 0 69 0 66 → 0 21 The concentration of sodium chloride may be calculated by multiplying the number on each tube by 1 5 and setting off two decimal places

6 Five ml of venous blood is collected with a dry syringe and needle and mixed in a bottle with 7 5 mg of heparin The blood is mixed and aerated well before use

7 Exactly 0 2 ml of blood is measured into each tube and the tubes are gently shaken to insure mixing

8 A control series of tubes must also be set up using normal blood

9 The racks containing the tubes are then placed in a refrigerator at 4° C for two to three hours

10 The tubes are examined and the point at which hemolysis begins and that at which it is complete are recorded The slightest trace of red color in the supernatant fluid indicates the destruction of the least resistant cells Complete hemolysis is indicated by a clear red solution and the absence of a residue in the bottom of the tube or of any cloudiness on gently shaking the tube

11 The tubes are then incubated for 24 hours at 37° C and reexamined

Calculations

Normal	Slight hemolysis	Complete hemolysis
Normal Blood	0 45-0 39%	0 33 0 30%

Note

The susceptibility of the red blood cell to rupture when placed in hypotonic solutions is determined primarily by the ratio of cell size to surface area The classic spherocyte has the smallest possible surface area Any increase in the content of this cell results in rupture As these cells behave as perfect osmometers water gains access to the cell in hypotonic solutions It is this added volume which brings about rupture

At the other end of the scale is the large flat cell of thalassemia or the flattened irregular cell of sickle cell anemia In both the surface area is increased relative to the cell size and more water may enter these cells without their rupture They are more resistant to hypotonic solutions than normal cells

Metabolic activity within the cell plays a role in the structural integrity of the cell also It is this feature that becomes important on incubation

References

1 Emerson C P Shen S C Ham T H and Castle W B The mechanism of blood destruction in congenital hemolytic jaundice J Clin Invest 26 1180 1947
2 Young L E Hereditary spherocytosis Am J Med 18 486 1955

SERUM BILIRUBIN

Reagents

1 Diazo Blank 15 ml concentrated HCl made up to one liter with distilled water

2 Solution A" 1 gm sulfanilic acid dissolved in 15 ml concentrated HCl and diluted to one liter with distilled water

3 Solution B 0 5 per cent solution of sodium nitrite (must be made fresh weekly)

4 Diazo Test Reagent 10 ml solution "A and 0 3 ml solution B" (must be made fresh daily)

5 Absolute methyl alcohol

Method

A INDIRECT

1 Two colorimeter tubes are set up as follows

Indirect Blank
a 5 0 ml absolute methyl alcohol
b 1 0 ml Diazo blank solution

Indirect Test
a 5 0 ml absolute methyl alcohol
b 1 0 ml Diazo test reagent

2 1 ml serum (or plasma) is diluted to 10 ml with distilled water and 4 0 ml of this mixture is added to each of the above tubes

3 Mix carefully by inversion taking care not to trap bubbles

4 Allow both tubes to stand 30 minutes and then read in the colorimeter using a filter #540 Tube #1 is used for the initial adjustment of the colorimeter

5 If the galvanometer reading is less than 10 both tubes are diluted immediately with 10 ml of 50 per cent methyl alcohol and read again The final answer in mg per 100 ml must then be multiplied by 2

B DIRECT

Method is the same as that for indirect except that distilled water is used instead of methyl alcohol and the sample is read against the blank exactly 60 seconds after the addition of the plasma or serum

2 Into the first tube is measured 4 8 ml of the sodium chloride solution into the second 4 6 ml and so on in amounts corresponding to the numbers on the tubes

3 To each tube there is added the amount of distilled water required to bring the total in each tube to 5 ml

4 The concentration of sodium chloride in each tube will be from left to right 0 72 per cent 0 69 0 66 → 0 21 The concentration of sodium chloride may be calculated by multiplying the number on each tube by 1 5 and setting off two decimal places

6 Five ml of venous blood is collected with a dry syringe and needle and mixed in a bottle with 7 5 mg of heparin The blood is mixed and aerated well before use

7 Exactly 0 2 ml of blood is measured into each tube and the tubes are gently shaken to insure mixing

8 A control series of tubes must also be set up using normal blood

9 The racks containing the tubes are then placed in a refrigerator at 4 C for two to three hours

10 The tubes are examined and the point at which hemolysis begins and that at which it is complete are recorded The slightest trace of red color in the supernatant fluid indicates the destruction of the least resistant cells Complete hemolysis is indicated by a clear red solution and the absence of a residue in the bottom of the tube or of any cloudiness on gently shaking the tube

11 The tubes are then incubated for 24 hours at 37° C and reexamined

Calculations

Normal	Slight hemolysis	Complete hemolysis
Normal Blood	0 45-0 39%	0 33 0 30%

Note

The susceptibility of the red blood cell to rupture when placed in hypotonic solutions is determined primarily by the ratio of cell size to surface area The classic spherocyte has the smallest possible surface area Any increase in the content of this cell results in rupture As these cells behave as perfect osmometers water gains access to the cell in hypotonic solutions It is this added volume which brings about rupture

At the other end of the scale is the large flat cell of thalassemia or the flattened irregular cell of sickle cell anemia In both the surface area is increased relative to the cell size and more water may enter these cells without their rupture They are more resistant to hypotonic solutions than normal cells

Metabolic activity within the cell plays a role in the structural integrity of the cell also It is this feature that becomes important on incubation

Method

1 All fecal material is collected for four days It should be kept in a dark bottle and weighed Record weight

2 Homogenize the feces in a Waring blender slowly adding distilled water in multiples of 100 ml as needed Usually 100 ml is adequate

3 Weigh out duplicate 10 gm samples of homogenized feces in paper cups Perform the following procedure in duplicate

4 Pour the sample into a dark bottle to which the freshly prepared 100 ml of 20% ferrous sulfate solution has been added

5 The paper cup is rinsed with a total of 290 ml distilled water to make sure all stool is transferred

6 Mix by shaking

7 100 ml of 10% NaOH is added slowly to the flask

8 Mix thoroughly

9 Incubate in dark for one hour or longer if the supernatant is not almost colorless

10 In order to determine the proper dilution to be used in the remainder of test a 2 ml portion of solution is now filtered and to it is added 2 ml Ehrlich's reagent and 6 ml saturated aqueous sodium acetate Depending on the intensity of the color formed from 4 to 50 ml of filtrate is used in the rest of the procedure

intense red color—4 ml filtrate
pale red—10 ml of filtrate
faint pink—25 ml of filtrate
no color—50 ml of filtrate

11 Filter the amount required and place in a separatory funnel Record the amount used If 4 or 10 ml samples are used make up to 25 ml with distilled water

12 Add 50 ml petroleum ether

13 Acidify with 5 ml glacial acetic acid

14 Shake well and allow to separate

15 Extract the aqueous portion twice again with 25 ml petroleum ether

16 Wash combined petroleum ether solution twice with 5 ml aliquots of distilled water

17 Add 2 ml Ehrlich's reagent and shake vigorously

18 Add 6 ml saturated aqueous solution of sodium acetate and shake The colored solution is drawn off into a graduated cylinder after complete separation

19 The petroleum ether is extracted again with Ehrlich's reagent followed by addition of sodium acetate and removal after vigorous shaking of the colored aqueous fraction

20 Repeat extractions until there is no further development of color in the aqueous solution

Calculation

The bilirubin concentration in mg per 100 ml of serum is obtained from the formula

$$x = \frac{2 - \log G}{6\,72} \times 100$$

x = bilirubin/100 ml serum
G = galvanometer reading

Normal Indirect (total)—below 1 0 mg %
Direct—below 0 4 mg %

Note

It is now known that the difference between the direct acting and the indirect type is the glucuronide conjugation of the former which renders it water soluble Conjugation of bilirubin with glucuronide is brought about in the liver by the enzyme transferase located in the microsomal fragment of the cells Uridine diphosphoglucuronic acid is the source of the glucuronide

Calibration of the galvanometer may be carried out using pure bilirubin as noted in the method described by Malloy and Evelyn (reference 1) A modification of the above method has recently been introduced (reference 2)

Reference

1 Malloy H T and Evelyn K A The determination of bilirubin with the photoelectric colorimeter J Biol Chem *119* 481 1937
2 O Hagen J E Hamilton T Le Breton E G and Shaw A E Human serum bilirubin An immediate method of determination and its application to the establishment of normal values Clin Chem *3* 609 1957

FECAL UROBILINOGEN

Reagents

1 Sodium acetate Add sodium acetate to distilled water until the solution is saturated Remove supernatant for use

2 Ehrlich s Reagent

0 7 gm para dimethyl aminobenzaldehyde
100 0 ml distilled water
150 0 ml concentrated HCl

3 20% ferrous sulfate

20 gm ferrous sulfate in 92 ml H_2O May require heating to go into solution

"Evans Blue" and the spectrophotometer J Clin Invest 16 301 1937
2 Granick S Iron metabolism Bull N Y Acad Med 30 81 1954
3 Miller E B Singer K and Dameshek W Use of the daily fecal output of the urobilinogen and the hemolytic index in the measurement of the hemolysis Arch Int Med 70 722 1942
4 Schwartz S Sborov V and Watson C J Studies of urobilinogen IV The quantitative determination of urobilinogen by means of the Evelyn photoelectric colorimeter Am J Clin Path 14 598 1944
5 Watson C J Schwartz S Sborov V and Bertie, E Studies of urobilinogen V A simple method for the quantitative recording of the Ehrlich reaction as carried out with urine and feces Am J Clin Path 14 605 1944
6 Watson C J and Hawkinson V Studies of urobilinogen VI Further experience with the simple quantitative Ehrlich reaction Corrected calibration of the Evelyn colorimeter with a Pontacyl Dye mixture in terms of urobilinogen Am J Clin Path 17 108 1947

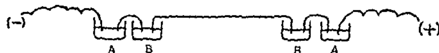
ELECTROPHORESIS

General

Electrophoresis has come to play an important role in clinical medicine Its aid in the study of the dysproteinemias and in sorting the various hemoglobinopathies is indispensable

The behavior of the protein molecule during electrophoresis depends on a number of complex and interrelated forces The protein molecules in the plasma and those in the red blood cell hemolysate may vary in size shape and their net electrostatic charge Electrophoretic analysis makes use of such variations to separate and classify these proteins The degree of resolution of these proteins obtained by electrophoresis is influenced by the environment and by the character of the electrical field

In most clinical laboratories electrophoresis is carried out using paper starch grain agar or starch gel as the supporting medium and an electric field supplied from a constant voltage power pack In the accompanying diagram the basic design of the commonly used apparatus is seen.



Boxes A usually contain a sodium chloride solution and boxes B a buffer solution The upporting medium going between the B boxes contains the same buffer A great many variations on this basic design have been published For an excellent and detailed discussion of these and related problems the reader is referred to the book *A Manual of Paper Chromatography and Paper Electrophoresis* (reference 1)

Apparatus of varied design may be obtained commercially

21 The colored solution is made to a convenient volume The color should be pink Record total volume

22 Read 10 ml of final solution in colorimeter using filter #565 against blank of 9 ml saturated sodium acetate and 3 ml Ehrlich s reagent

Calculation

$$\frac{500\ (H\ O\ \ FeSO_4\ \text{and}\ NaOH)}{10\ (\text{gm of stool})} \times \frac{\text{Vol of final dilution}}{\text{Vol of filtrate used}} \times$$

$$\%\ \text{of color standard} \times \frac{\text{Wt of four day collection}}{100} \times$$

$$\frac{1}{\text{No of days of collection}} = \text{mg of urobilinogen per day}$$

Normal 156–195 mg fecal urobilinogen

Note

Normally 6 5 gm of hemoglobin is destroyed daily and this should result in 156 to 195 mg urobilinogen It is recognized that the measure of fecal urobilinogen is not an accurate measure of daily hemoglobin breakdown There are a number of sources of error Hemoglobin is not the sole source of bile pigment as myoglobin catalase cytochromes and peroxidases may contribute Dietary porphyrins do not alter the fecal urobilinogen as they are not broken down in the intestinal tract (reference 2) Constipation diarrhea the use of antibiotics (reducing intestinal flora) and incomplete collections will all add to inaccuracies However an increase in the fecal urobilinogen output indicates an increase in blood destruction

Since there are marked differences in fecal urobilinogen dependent on the circulating mass of hemoglobin a formula was devised to take this into account (reference 3)

Hemolytic Index =

$$\frac{\text{average daily (of 4 days) output of fecal urobilinogen (mg)} \times 100}{\text{total hemoglobin (gm 100 ml)} \times \frac{\text{total blood volume*}}{100}}$$

Normal 11 1–20 8 mg

N B

To standardize the colorimeter a mixture of Pontacyl Dyes is available (DuPont) The method is given by Watson and Hawkinson (reference 6)

References

1 Gibson J G II and Evans W A Jr Clinical studies of the blood volume I Clinical application of a method employing the azo dye

* May use expected total blood volume (reference 1)

Buffers

Barbiturate Buffer 1 84 gm diethylbarbituric acid (barbital) and 10 30 gm sodium barbital are made up to one liter in distilled water The ionic strength of this buffer is 0 05 and the pH is 8 6 It is excellent for runs of six to eight hours duration

For runs of 10 to 12 hours duration better resolution is obtained using buffer of 0 1 ionic strength 2 76 gm diethylbarbituric acid (barbital) and 15 45 gm sodium barbital are made up to one liter in distilled water

Stains Used in Connection with Paper Electrophoresis

Bromphenol Blue staining is most commonly employed

0 1 gm bromphenol blue

50 gm zinc sulfate

50 ml glacial acetic acid

The dry paper is immersed in the dye solution for 12 to 16 hours It is removed and rinsed three times in 2% acetic acid and finally in a solution of 10% acetic acid and 2% sodium acetate It is then blotted and dried in the oven

STARCH GEL ELECTROPHORESIS

The use of starch gel as a supporting medium for electrophoresis has gained widespread acceptance since its introduction by Smithies (reference 1) Excellent resolution of serum and red blood cell hemolysate proteins is obtainable The gels take a wide variety of stains In most instances they may be stored in large test tubes without loss or distortion of staining characteristics

Preparation of Starch Gels (Smithies Modified Slightly)

1 200 ml of gel buffer is mixed with 25 gm hydrolyzed starch *

2 The mixture is gently heated over a Bunsen burner in a flask to which a negative pressure may be applied from either a vacuum pump or a water pump

3 Heating is continued as the gel becomes more viscid and finally thin once again As the gel becomes less viscid it will begin to clear At this point negative pressure is applied and the liquid allowed to degas vigorously The length of time allowed the mixture to degas should be constant as it affects the water content of the gel and variations will affect the constancy of the results from day to day

4 The gel is poured into plastic boats (reference 1) and some of which the pH is later determined into a small beaker

5 Filter paper wicks are cut so that they may be inserted into the gel

* Hydrolyzed starch may be obtained from the Connaught Laboratories Toronto

Reference

1 Block R J Durrum E L and Zweig G A Manual of Paper Chromatography and Paper Electrophoresis 2nd Ed Academic Press Inc New York 1958

PREPARATION OF RED BLOOD CELL HEMOLYSATE FOR ELECTROPHORESIS

1 Fresh anticoagulated (heparin or oxalate) blood is centrifuged to separate the plasma from the cells

2 The plasma is discarded and the cells washed three times with 0 85% NaCl and centrifuged between each washing The supernatant is discarded

3 1 2 to 1 8 volumes of distilled water is then added to the cells and the solution shaken vigorously

4 0 3 to 0 4 ml of toluene is then added and again the mixture is agitated

5 Filter and adjust the hemoglobin solution to approximately 5 gm per 100 ml with distilled H_2O

6 Refrigerate the filtrate if not used immediately

Note

Should it be desirable not to add toluene the ghosts may be removed by centrifugation in a high speed head

PREPARATION OF SERUM FOR ELECTROPHORESIS

1 Fresh blood is obtained in a dry syringe and allowed to drip from the syringe into a clean dry test tube after removal of the needle

2 After clot retraction takes place the serum is removed and placed in the refrigerator until used

PAPER ELECTROPHORESIS

Paper has been widely used as a supporting medium in electrophoresis Usually a good grade of paper such as Whatman 3 MM is used though heavier paper may be used when larger volumes are to be run Most commonly the paper is enclosed in a plastic box or sandwiched between glass plates to prevent drying Paper may be employed on a horizontal plane or may hang from a central supporting rod in the shape of an inverted V

After each run it is important that the wick ends are cut off immediately to prevent shifting of the position the proteins achieved during the run as the wicks lose fluid The paper should be dried rapidly in a horizontal position in an oven at 100 C for 20 to 30 minutes Drying the proteins brings about their denaturation and fixes them on the paper Staining may then be carried out if the proteins are not pigmented It is usually unnecessary to stain hemoglobin as its position is clearly visible

BRIDGE. Citric acid 0 1 M and Na_2HPO_4 0 2 M are mixed in the amounts needed to provide proper volume and pH and used full strength

Outer Boxes

NaCl is made up to the same molarity as that of the buffer being used

Protein Stain for Use with Starch Gel Electrophoresis

Amidoschwarz 10B A solution of methyl alcohol distilled water and glacial acetic acid is made up 50 50 10 v/v Amidoschwarz 10B is added until the solution is saturated The same solution without the dye is used as a wash

The cut strips of gel are immersed in the dye solution for 30 seconds and then removed to the wash solution Three washes generally clear the gel enough to allow definition of protein fractions Further clearing will take place if gel is left over night in the solution

Gels may be stored in the same solution

Reference

1 Smithies O G Zone electrophoresis in starch gels Group variations in the serum proteins of normal human adults Biochem J 61 629 1955

DETECTION OF FETAL HEMOGLOBIN BY THE ALKALI DENATURATION TECHNIQUE

(References 1 and 4)

Reagents

1 *N/12 KOH* or *NaOH (pH 12 7)* in paraffin lined bottle—kept in refrigerator

2 Precipitating solution 800 ml 50% saturated $(NH_4)_2SO_4$ plus 2 ml 10 N HCl

Method

1 Centrifuge fresh anticoagulated blood (heparin or oxalate) and discard plasma

2 Wash remaining cells three times with three or four volumes of normal saline discarding the washings

3 Add 1 2 to 1 8 volumes of distilled H_2O Shake vigorously for three minutes

4 Add 0 4 volumes of toluene and again shake for three minutes

5 Centrifuge at 3000 r p m for 20 minutes and discard upper two layers

at either end A piece of thin polyethylene sheeting is cut to fit the surface then placed over the boat and pressure applied evenly Excess gel will be extruded

6 After cooling the application of protein is made by cutting the gel with a razor blade and inserting a piece of filter paper carrying the substance to be examined The filter paper should be slightly smaller in both dimensions than the cross section of the gel

7 The plastic is replaced and the wicks dipped into the boxes (B) containing the buffer and filter paper wicks arranged to bridge from these boxes to those containing the electrodes (A)

8 Current is applied at 5 ma /boat and 5 6 volts/cm for six to eight hours

9 After six to eight hours has elapsed the gels are loosened from their boats by running a spatula between the gel and the edge of the boat They may then be gently removed

10 The gels are then placed in a plastic trough the width of which is the same as that of the gel the length somewhat greater than that of the gel and the height of the sides exactly half that of the thickness of the gel By placing half of a double edged razor blade across the trough the gel may be easily cut lengthwise into two identical halves The inner surfaces are stained and examined It is the inner surface of the gel on which protein migration is least affected by distortion

Buffers

A number of buffers have been employed in starch gel electrophoresis We have used Sorensens phosphate buffer phosphate–citric acid buffer and borate buffer with success The gels behave well at low and high pH The molarity of the gel buffer should be between 0 02 and 0 03 Since the starch itself alters the pH of the buffer the final pH of the gel must be determined after it has cooled sufficiently

The bridge buffer should be at the same pH as that of the gel and approximately 0 3 M

Borate Buffer GEL 1 42 gm H_3BO_3 is dissolved in a liter of distilled water The desired pH is obtained by the addition of NaOH solution

BRIDGE 18 55 gm of H_3BO_3 is dissolved in a liter of distilled water The desired pH is obtained by the addition of NaOH solution We have found it convenient to add 40% NaOH dropwise until the desired pH is reached

Sorensen s Buffer GEL M/15 Na HPO_4 and M/15 KH PO_4 are mixed in proportions needed to obtain the proper pH and the solution then diluted v/v with distilled water

BRIDGE M/15 Na HPO_4 and M/15 KH PO_4 are mixed in proportions needed to obtain desired pH and used full strength

Citric Acid Buffer GEL Citric acid 0 1 M and Na HPO_4 0 2 M are made and mixed in proportions needed to obtain the desired pH 20 ml of the solution is then made up to 200 ml with distilled water

2 Itano H A Bergren W R and Sturgeon P The abnormal human hemoglobins Medicine 35 121 1956
3 Jacob G F and Raper A B Hereditary persistence of foetal haemoglobin production and its interaction with sickle cell trait Brit J Haemat 4 138 1958
4 Singer K Chernoff A I and Singer L Studies on abnormal hemoglobins 1 Their demonstration in sickle cell anemia and other hematologic disorders by means of alkali denaturation Blood 6 413 1951
5 White J C and Beavan G H Foetal haemoglobin Brit Med Bull 15 33 1959

BLEEDING TIME

I DUKE METHOD

Apparatus

1 Sterile Bard Parker blade
2 Filter paper 8 inches in diameter

Method

1 Make a skin puncture in the lobe of the ear
2 Blot with filter paper (without touching the skin) every 1/2 minute until bleeding stops

Calculation

Normal 1–3 minutes

II IVY METHOD

Apparatus

1 Spring lancet
2 Filter paper
3 Sphygmomanometer

Method

A sphygmomanometer cuff is fixed about the arm and the pressure raised to 40 mm Hg With a spring lancet the blade set at 2 5 mm three wounds are made on the extensor surface of the forearm care being taken to avoid scars and superficial veins

Using three filter paper discs each wound is gently blotted every 15 seconds until bleeding stops

The average of the time taken for bleeding to stop is computed by adding the total number of blots on the papers and dividing by twelve

6 Filter the clear red solution and adjust to a concentration of 9 to 11 gm per 100 ml hemoglobin by adding appropriate amounts of distilled H_2O (Solution A)

7 Place 1 6 ml of the alkali reagent in a serologic test tube and keep in a water bath at 20° C for several minutes

8 0 1 ml of the hemoglobin solution is added with a blow out pipette The pipette is rinsed several times and the tube gently shaken for 10 seconds

9 Start stop watch at the moment the hemoglobin solution is introduced and in exactly one minute add 3 4 ml of the precipitating solution

10 Invert six times and immediately filter through a double layer of filter paper into a clean test tube (Solution B)

Calculation (References 1 and 4)

The amount of fetal hemoglobin in the original hemoglobin solution is determined by comparing the optical density of solution A with solution B in a colorimeter at 540 millimicrons Solution B is a 1 50 dilution of solution A and this dilution factor must be considered when comparing the two hemoglobin solutions

If a Beckman spectrophotometer is used 0 02 ml (Sahli pipette) of solution A may be diluted with 4 ml of distilled water (Solution C) and its optical density determined without dilution The percentage of fetal hemoglobin is then determined

$$\frac{1/4 \text{ optical density solution B}}{\text{optical density solution C}} \times 100 = \% \text{ Fetal hemoglobin}$$

Comment

The resistance of fetal hemoglobin to alkali denaturization is well known However the alkali resistant fraction in this test is a mixture of undenatured fetal hemoglobin and dissolved denatured hemoglobin The precipitated material is also a mixture consisting of denatured hemoglobin and a small amount of adsorbed, undenatured fetal hemoglobin (reference 2)

As normal adult bloods give one minute residues between 0 5 to 1 7% only those above 2% are considered as positive evidence of the presence of fetal hemoglobin (reference 4) The presence of fetal hemoglobin indicates a continuation of the production of the pigment beyond the usual age limit or a reactivation of its production The mechanism of its formation and its significance is not clear (reference 5) It has been found in the blood of patients with hereditary hemoglobinopathies thalassemia and pernicious anemia It is not present in the acquired hemolytic anemias (reference 5) There has been a suggestion that fetal hemoglobin may persist to adult life unassociated with any other hematologic abnormality (reference 3)

References

1 Chernoff A I The human hemoglobins in health and disease New England J Med 253 322 365 416 1955

It has been noted by Macfarlane (reference 2) that there is a linear increase in the degree of clot retraction as packed cell volume decreases The relationship between decreased clot retraction and the presence of jaundice and pneumonia is not clear The abnormal proteins present in myeloma are known to interfere with clotting and may affect clot retraction as well

References

1 Ackroyd J F A simple method of estimating clot retraction with a survey of normal values and the changes that occur with menstruation Clin Sc 7 231 1948-49
2 Macfarlane R G A simple method for measuring clot retraction Lancet 1 1199 1939

THE TOURNIQUET TEST OF CAPILLARY FRAGILITY

Method (Reference 4)

1 A small circle 2 5 cm in diameter is drawn on the inner aspect of the forearm about 4 cm below the crease of the elbow Any skin blemishes which might be confused with petechiae are marked

2 A manometer cuff is elevated to midway between diastolic and systolic pressure and held for five to ten minutes

3 Five minutes after the pressure is released the number of petechiae within the circle are counted

4 If there is excessive discomfort or if petechiae appear the test is terminated It should not be repeated in under seven or eight days

Calculation

Normal 0-10
Marginal 10-20
Abnormal 20+

Note

This procedure first described by Hess in 1914 (reference 3) provides a measure of the ability of the vascular endothelium to withstand trauma and recover from it It is thought that small breaks occur frequently in the capillary walls and that these breaks are normally plugged by platelets and perhaps by fibrin The test when positive indicates a defect in the vascular endothelium its supporting structures or perhaps some plasma factor or cellular element important to the maintenance of vascular integrity Marked thrombocytopenia for example may result in a positive test

Tests of capillary resistance are not entirely satisfactory since there is considerable variation in the results on the same person at different times and indeed in different arms (reference 2)

Calculation

Normal 3–4 minutes (some accept 7 minutes)

Note

This test was originally described by Duke in 1910 and depends on the normal function of small vessels as well as the presence of adequate numbers of normal platelets

References

1 Duke W W The relation of blood platelets to hemorrhagic disease J A M A *55* 1185 1910
2 Ivy A C Shapiro P F and Melnick P The bleeding tendency in jaundice Surg Gynec & Obst *60* 781 1935

CLOT RETRACTION

Apparatus

1 Graduated centrifuge tubes (must read to 0 1 ml)
2 Wire of diameter 1 to 1 5 *mm* so coiled that there are six turns to the inch Spiral should be two inches long

Method

1 Something over 5 ml of blood is drawn and after removing the needle the blood is allowed to flow gently into the centrifuge tube to the 5 ml mark
2 The wire spiral is put in place in the tube
3 The tube is then incubated at 37° C in a waterbath After the blood has clotted firmly the tube is left undisturbed for an additional hour
4 The wire spiral is then removed allowing for drainage
5 The volume of the remaining serum and cells expressed from the clot is read directly in the tube and the volume expressed as a percentage of the original 5 ml

Calculation

Normal Retraction between 48 and 64% with mean of 54 7%

Note

The rate and the completeness of clot retraction are affected by
a The quantity and quality of the platelets
b The red blood cell volume
c The presence of such disorders as myeloma pneumonia and jaundice

INDIRECT PLATELET COUNT

Method

1 Make a blood smear immediately from needle after venipuncture or from finger puncture
2 Stain with Wright's stain
3 Count the number of platelets per 1000 red cells
4 Determine red blood cell count
5 $\frac{\text{Platelets/1000 RBC} \times \text{RBC/cu mm}}{1000}$ = platelets/cu mm

Calculation

Normal 250 000-600 000 per cu mm

Note

Quantitative and perhaps qualitative changes in the platelets may be of importance in coagulation A quantitative measure of the platelets may be obtained by a direct chamber count or indirectly by relating the number of platelets to the number of red blood cells present on a blood film A markedly diminished platelet count indicates a failure to produce platelets at a sufficient rate and/or their increased destruction A quantitative decrease in platelets is known as thrombocytopenia

References

1 Bray W C Synopsis of Clinical Laboratory Methods 3rd Ed C V Mosby Co St Louis 1946 p 113
2 Brecher G and Cronkite E P Morphology and enumeration of human blood platelets J Appl Physiol 3 365 1950

COAGULATION TIME

Apparatus

1 Four test tubes 2½ by ⅜ inches
2 Waterbath at 37 C

Method

1 Venous blood is drawn into a clean dry all glass syringe with a tightly fitting sharp 18 gauge needle
2 Detach the needle and gently deliver 1 ml volumes of blood into each of four 2½ by ⅜ inch test tubes If possible keep tubes in waterbath at 37 C
3 A stop watch is started at the moment the blood first enters the syringe

A small suction cup as described by Dalldorf (reference 1) may also be used to determine capillary resistance

References

1 Dalldorf G A sensitive test for subclinical scurvy in man Am J Dis Child 46 794 1933
2 Greene D Evaluation of the capillary resistance test in the diagnosis of subclinical scurvy J A M A 103 4 1934
3 Hess A F and Fish M Infantile scurvy The blood the blood vessels and the diet Am J Dis Child 8 385 1914
4 Wright I S and Lilienfeld, A Pharmacologic and therapeutic properties of crystalline vitamin C Arch Int Med 57 241 1936

PLATELET COUNT

DIRECT PLATELET COUNT WITH PHASE MICROSCOPE

Reagent and Apparatus

1 Diluent for platelet counts—1 per cent ammonium oxalate in distilled H_2O
2 Phase contrast microscope
3 Red blood cell counting chamber and pipettes

Method

1 Make a deep finger puncture Discard the first drop of blood then draw blood to 1 mark on the red cell pipette avoiding touching the end of the pipette on the skin Draw diluent to 101 mark making a 1 100 dilution Using another pipette make a duplicate preparation

2 Rotate the pipettes to mix the contents well then place in a mechanical shaker for 20 minutes

3 Discard ⅓ of contents of one pipette and fill both sides of the red cell counting chamber Repeat with second pipette and chamber

4 Let both chambers stand for 15 minutes in a covered Petri dish A piece of wet cotton placed in each dish will prevent evaporation

5 Counting is done using 43X dry phase *contrast* objective and counting chamber with a flat bottom Platelets will be seen to have a faint halo Shadows of red cells may be seen in the background

Calculation

Count at least 300 platelets

$$\frac{\text{Total no of platelets counted} \times 10 \times \text{dilution}}{\text{no of 1 mm sq counted}} = \text{platelets per cu mm}$$

Counts must be completed within 8 hours after collection of the blood

b Centrifuge at 1500 r p m for 10 minutes
c Separate platelet containing plasma into silicone centrifuge tubes
d Centrifuge at 3000 r p m for 15 minutes
e Clear supernatant is used as substrate plasma
f Platelets deposited in button at bottom of tube are fragmented with wooden stick and washed twice with 0 85% NaCl
g Resuspend in 0 85% saline to ⅓ to ⅕ original volume of plasma

Method

1 Measure 0 1 ml amounts of substrate plasma in Lee and White clotting tubes and place in waterbath at 37 C

2 Pipette into a Lee and White clotting tube

0 3 ml 1/5 dilution of normal adsorbed plasma
0 3 ml 1/10 dilution of normal serum
0 3 ml 1/100 dilution of chloroform extract of brain

3 Place in waterbath at 37 C

4 Add 0 3 ml M/40 CaCl to the mixture and start stop watch Agitate with 0 1 ml pipette

5 At one minute add 0 1 ml of initial mixture and 0 1 ml M/40 CaCl to a tube of substrate plasma Start stop watch and time clot

6 Repeat at minute intervals until minimum clotting time is recorded Repeat at 12 minutes to confirm minimum clotting time

7 Repeat test substituting in turn patient s serum and plasma for normal and then both the patient s plasma and serum together

8 The test may be done using a suspension of platelets from normal control and from the patient which are substituted in turn for the brain extract

Note

As has been noted thromboplastin activity is thought to develop in the intrinsic (blood) system when there are present in the incubation mixture antihemophilic factor plasma thromboplastin component plasma thromboplastin antecedent Stuart factor platelets Factor V and ionized calcium The thromboplastin generation test provides a method by which a specific deficiency of one of these factors may be discovered

Normal plasma treated with $Al(OH)_3$ provides AHF PTA Factor V and Hageman factor but does not contain Factor VII Stuart factor or PTC Serum should contain Stuart factor Factor VII PTA PTC and Hageman factor while Factor V and AHF which are consumed in clotting are lacking (Factor VII is not essential for development of thromboplastin activity in the intrinsic system) If the three reagents $Al(OH)_3$ adsorbed plasma normal serum and platelets or brain extract are incubated in the presence of CaCl there is developed a labile but powerful thromboplastin activity The efficiency of the thromboplastin activity is then measured by

4 The four tubes are tilted in turn at half minute intervals until each can be tilted through an angle greater than 90 degrees without spilling

5 The clotting time of each tube is measured separately and the average of the four times reported

Calculation

Normal 5 to 10 minutes As the normal range for the Lee and White test varies in different laboratories and from time to time in the same laboratory a normal control should be run once a week

Note

The whole blood coagulation time was described late in the nineteenth century but was standardized by White and Lee in 1913 (reference 1) The test is a measure of the adequacy of the various factors which take part in the intrinsic system to bring about coagulation The technique must be scrupulous in order that admixture with tissue juices does not occur during collection of the blood The test is insensitive and may fail to expose serious defects in coagulation It may for example be normal in the presence of severe AHF deficiency

Reference

1 White P D and Lee Roger I A clinical study of the coagulation time of blood Am J M Sc *145* 495 1913

THE THROMBOPLASTIN GENERATION TEST

Reagents

1 Normal adsorbed plasma diluted 1/5 with 0 85% NaCl This must stand for at least 10 minutes

2 Patient s adsorbed plasma diluted 1/5 with 0 85% NaCl This must stand for at least 10 minutes

3 Normal serum diluted 1/10 with glyoxaline buffer This must stand for 1 hour

4 Patient s serum diluted 1/10 with glyoxaline buffer This must stand for 1 hour

5 Chloroform extract of brain diluted 1/100 with saline

6 M/40 CaCl

7 Substrate plasma

8 Platelet supsension

NORMAL SUBSTRATE AND PLATELET SUSPENSIONS

a Twenty ml of whole normal blood is collected into two silicone treated 10 ml graduated centrifuge tubes each of which contains 1 ml of 3 8% sodium citrate

b Centrifuge at 1500 r p m for 10 minutes
c Separate platelet containing plasma into silicone centrifuge tubes
d Centrifuge at 3000 r p m for 15 minutes
e Clear supernatant is used as substrate plasma
f Platelets deposited in button at bottom of tube are fragmented with wooden stick and washed twice with 0 85% NaCl
g Resuspend in 0 85% saline to ⅓ to ½ original volume of plasma

Method

1 Measure 0 1 ml amounts of substrate plasma in Lee and White clotting tubes and place in waterbath at 37° C

2 Pipette into a Lee and White clotting tube
0 3 ml 1/5 dilution of normal adsorbed plasma
0 3 ml 1/10 dilution of normal serum
0 3 ml 1/100 dilution of chloroform extract of brain

3 Place in waterbath at 37° C

4 Add 0 3 ml M/40 CaCl to the mixture and start stop watch Agitate with 0 1 ml pipette

5 At one minute add 0 1 ml of initial mixture and 0 1 ml M/40 CaCl to a tube of substrate plasma Start stop watch and time clot

6 Repeat at minute intervals until minimum clotting time is recorded Repeat at 12 minutes to confirm minimum clotting time

7 Repeat test substituting in turn patient s serum and plasma for normal and then both the patient s plasma and serum together

8 The test may be done using a suspension of platelets from normal control and from the patient which are substituted in turn for the brain extract

Note

As has been noted thromboplastin activity is thought to develop in the intrinsic (blood) system when there are present in the incubation mixture antihemophilic factor plasma thromboplastin component plasma thromboplastin antecedent Stuart factor platelets Factor V and ionized calcium The thromboplastin generation test provides a method by which a specific deficiency of one of these factors may be discovered

Normal plasma treated with $Al(OH)_3$ provides AHF PTA Factor V and Hageman factor but does not contain Factor VII Stuart factor or PTC Serum should contain Stuart factor Factor VII PTA PTC and Hageman factor while Factor V and AHF which are consumed in clotting are lacking (Factor VII is not essential for development of thromboplastin activity in the intrinsic system) If the three reagents $Al(OH)_3$ adsorbed plasma normal serum and platelets or brain extract, are incubated in the presence of $CaCl_2$ there is developed a labile but powerful thromboplastin activity The efficiency of the thromboplastin activity is then measured by

placing aliquots of the incubation mixture in a tube containing a substrate of normal platelet free citrated plasma Normally coagulation will occur within 10 seconds By serially substituting the patient s serum $Al(OH)_3$ treated plasma or platelets in this test specific deficiencies of the early phase of coagulation may be revealed

It is important to note that though the substrate plasma contains factors that may be missing from test fractions the time that would be required for them to react in the substrate tube would be in excess of the 10 second limit defining normality in this test The development of thromboplastin activity normally requires 3 to 5 minutes What is measured when the incubation mixture is transferred to the second tube containing the substrate plasma is the rate of conversion of prothrombin to thrombin and the subsequent conversion of fibrinogen to fibrin If the substrate plasma is normal the end point is dependent solely on the amount of thromboplastin activity that was developed during the incubation period The fibrinogen and prothrombin in the substrate are constant while the potential variables are in the incubation mixture The variables may then be separated by performing the classic one stage prothrombin time test

Reference

1 Biggs R and Douglas A S The thromboplastin generation test J Clin Path 6 23 1953

ONE STAGE PROTHROMBIN TIME

Reagents

1 Normal plasma Obtain 4 5 ml blood by venipuncture and immediately add to tube containing 3 8% sodium citrate Centrifuge at 1800 r p m for 10 minutes and withdraw clear supernatant plasma

2 Patient s test plasma Obtain as above

3 Brain thromboplastin Prepared from lyophilized acetone extracted rabbit brain available commercially

4 M/40 $CaCl_2$

Method

1 Pipette into a clotting tube
   0 1 ml normal plasma
   0 1 ml brain thromboplastin

2 Place tubes in waterbath at 37° C for one to two minutes

3 Add 0 1 ml M/40 CaCl trigger stop watch and time the clot

4 Repeat with patient s plasma

5 The test is usually done in duplicate and the results expressed as a ratio of the normal

Note

This test described by Quick in 1935 was designed to measure prothrombin in the blood Brain was supplied as a thromboplastin progenitor and fibrinogen and Ca^{++} were constant factors When it was first introduced it was felt that prothrombin was the only variable It is now recognized that Factor VII Factor V and Stuart factor are also important to the coagulation of fibrinogen under these conditions A deficiency of any one will affect the prothrombin time as will a deficiency of fibrinogen The test has been and still is extremely useful in the study of coagulation defects For the control of Dicumarol therapy many prefer Owren s modification (reference 1) In order to determine quantitatively the prothrombin concentration a two stage procedure may be used (reference 5)

References

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MODIFIED THROMBOPLASTIN GENERATION TEST

Reagents

1 Normal adsorbed plasma diluted 1/5 with 0 85% NaCl This must stand for at least 10 minutes

2 Normal serum diluted 1/10 with glyoxaline buffer This must stand for one hour

3 Chloroform extract of brain diluted 1/100 with saline

4 M/40 $CaCl_2$

5 Substrate normal plasma

a Ten ml of whole normal blood is collected in a silicone treated centrifuge tube containing 1 ml of 3 8% sodium citrate

b Centrifuge at 300 r p m for 15 minutes

c Use clear supernatant plasma

6 Substrate test plasma Obtain whole blood from patient and prepare as in 5 above

Method

1 Measure 0 1 ml amounts of substrate normal and test plasmas in Lee and White clotting tubes and place in waterbath at 37° C

2 Pipette into Lee and White clotting tube

0 3 ml 1/5 dilution of normal adsorbed plasma

0 3 ml 1/10 dilution of normal serum

0 3 ml 1/100 dilution of chloroform extract of brain

3 Place in waterbath at 37° C

4 Add 0 3 ml M/40 $CaCl_2$ to the mixture and start stop watch Agitate with 0 1 ml pipette

5 At the end of 4 6 and 8 minutes add 0 1 ml of M/40 $CaCl_2$ and 0 1 ml of incubation mixture to both the test and normal plasma Start stop watch and time the appearance of clot in each tube

Note

If the fibrinogen content of the substrate plasmas are normal and there is no anticoagulant present the rate of clot formation will depend on pro thrombin alone Factor VII which is essential for the normal evolution of thromboplastic activity in the Quick one stage prothrombin time test is not essential to this modification of the thromboplastin generation test This test then may be used to differentiate between prothrombin deficiency and Factor VII deficiency (references 1 and 2)

References

1 Hougie C Barrow E M and Graham J B Stuart clotting defect I Segregation of an hereditary hemorrhagic state from the hetero geneous group heretofore called stable factor" (SPCA procon vertin Factor VII) deficiency J Clin Invest 36 485 1957

2 Raccuglia G Duff I F and Bethell F H The detection of defects of first stage of coagulation J Lab & Clin Med 53 789 1958

THROMBIN TIME AND TITER

Reagents

1 Normal and patient s platelet free citrated plasma Whole citrated blood spun at 2000 r p m for 15 minutes will provide plasma sufficiently free of platelets

2 Thrombin (human thrombin or commercially available bovine throm bin)

3 Lee and White clotting tubes

Method

1 0 5 ml of platelet free citrated plasma from both normal and patient is serially diluted in doubling dilutions from 1/2 to 1/128

2 To each tube is added 0 1 ml human thrombin (56 units/ml) or bovine thrombin

3 The thrombin titer is the highest dilution containing a visible clot Normal titer 1/64–1/128

4 The thrombin time may be obtained by ascertaining the clotting time of the 1/2 dilution This should be determined for the normal and the patient

Note

If bovine thrombin is used in this test lower thrombin titers and longer clotting times are to be expected Bovine thrombin will frequently be available in the operating room as a hemostatic agent This may be made up to 100 units/ml in saline The thrombin concentration should be so adjusted as to give a clotting time for normal plasma of 10 to 12 seconds The test presented is a modification (reference 2) of one presented by Schneider (reference 1)

References

1 Schneider C L Rapid estimation of plasma fibrinogen concentration and its use as a guide to therapy of intravascular defibrination Am J Obst & Gynec 64 141 1952

2 Sharp A A Howie B Biggs R and Methuen D T Defibrination syndrome in pregnancy Value of various diagnostic tests Lancet 2 1309 1958

FIBRINOGEN ASSAY

Reagents

1 Half saturated solution of ammonium sulfate (Dissolve 26 gm $(NH_4)_2SO_4$ in 50 ml distilled water and then make to 100 ml with distilled H_2O)

2 Citrated plasma from patient and normal control

3 Esbach s centrifuge tubes (Hopkins type of vaccine tube Gallenkamp)

Method

1 Place 2 ml of patient s plasma in one modified Esbach centrifuge tube and 2 ml of control plasma in another

2 Add 2 ml half saturated ammonium sulfate to each of the tubes

3 Mix and allow to stand at room temperature for 10 minutes
4 Centrifuge at 2000 r p m for 10 minutes and compare volume of precipitate

Calculation

$$\frac{\text{Vol of patient's precipitate} \times 100}{\text{Vol of normal precipitate}} = \% \text{ of normal fibrinogen}$$

Note

This test is designed to estimate quickly the quantity of fibrinogen It cannot be relied upon to determine accurately the extent of "effective" fibrinogen deficiency in surgical and obstetrical defibrination since the fibrinogen (or precipitate) measured in these cases may not be physiologically active A thrombin titer or time should be used as well

Reference

1 Biggs R and Macfarlane R G Human Blood Coagulation and Its Disorders 2nd Ed Charles C Thomas Springfield Ill 1957

IMIDAZOLE BUFFER

Reagents

1 Imidazole (glyoxaline)
2 0 1 N HCl
3 Distilled water

Method

1 Dissolve 1 72 gm imidazole in 90 ml of 0 1 N HCl
2 Dilute with water to 100 ml
3 pH should be 7 25

Note

This is an excellent buffer for use in coagulation studies It is isosmotic with 0 9% NaCl

Reference

1 Mertz E T and Owen C A Imidazole buffer Its use in blood clotting studies Proc Soc Exper Biol & Med *43* 204 1940

ADSORPTION OF PLASMA

Plasma (or serum) may be adsorbed with a number of inorganic precipitating agents Most commonly used are $BaSO_4$ and $Al(OH)_3$

Use of $BaSO_4$

$BaSO_4$ must be treated to remove very fine particles which would otherwise remain in the plasma despite centrifugation. One method (reference 1) of preparing it is as follows:

1. One pound of x-ray quality $BaSO_4$ is suspended in 4000 ml. of 0.005 M trisodium citrate $(Na_3C_6H\,O\,H\,O)$ overnight.
2. Pour off supernatant and discard.
3. Resuspend in another 4000 ml. of 0.005 M trisodium citrate overnight.
4. Collect sediment on filter paper and dry in hot air oven.
5. Powder the dry material.

Use of $Al(OH)_3$

$Al(OH)_3$ may be prepared by suspending 1 gm. of alumina gel in 4 ml. of water (reference 1).

To adsorb a small amount of plasma:

1. To 1 ml. plasma add 0.1 ml. $Al(OH)_3$. Mix and incubate at 37° C. for two minutes.
2. Centrifuge at 2000 r.p.m. for seven minutes.
3. Remove and save supernatant.

For larger amounts:

1. To 1000 ml. plasma add 50 ml. $Al(OH)_3$. Mix and incubate at 37° C. for 15 minutes.
2. Centrifuge at 2000 r.p.m. for seven minutes.
3. Remove and save supernatant.

Reference

1. Biggs, R., and Macfarlane, R. G.: Human Blood Coagulation and Its Disorders. 2nd Ed. Charles C Thomas, Springfield, Ill., 1957.

DETECTION OF GLUTATHIONE INSTABILITY BY COMPARING HEINZ BODY FORMATION IN VITRO

Reagents

1. Buffer solution pH 7.6

    M/15 KH_2PO_4 1.3 parts
    M/15 Na_2HPO_4 8.7 parts
    200 mg. glucose/100 ml.

2. Acetylphenylhydrazine solution

    100 mg. acetylphenylhydrazine in 100 ml. of buffer solution

3 Crystal violet solution

2 gm crystal violet in 100 ml of 0 73% NaCl solution Shake 5 minutes and filter To the filtrate is added an equal volume of 0 73% NaCl Stable at room temperature at least five months

Method

1 Mix 0 1 ml heparinized blood (7 5 mg heparin/5 ml blood) and 2 0 ml acetylphenylhydrazine solution Blood must be used within one hour of the time sample is drawn Aerate suspension by blowing air through pipette two or three times

2 Incubate in uncapped tube in waterbath at 37° C for two hours

3 Aerate suspension again

4 Incubate for a second two hour period at 37° C

5 Agitate suspension and then mix a small drop of the suspension with a slightly larger drop of crystal violet solution on a microscope slide and cover with cover slip

6 After 5 or 10 minutes determine the percentage of cells having five or more Heinz bodies Field should be selected in which cells are not crenated or overlapping and in which there is no hemolysis

Comment

Normal (insensitive cells) 0–28%
Abnormal (sensitive cells) 45–98%

Reference

1 Beutler E Dern R J and Alving A S The hemolytic effect of primaquin J Lab & Clin Med *45* 40 1955

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INDEX

Numbers in *italic type* refer to illustrations. As used in this index, the abbreviation vs means differential diagnosis.